

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :  C12N 15/11, C12Q 1/68, A61K 48/00		A1	(11) International Publication Number: <b>WO 98/02539</b>
(21) International Application Number: <b>PCT/US97/12343</b>		(43) International Publication Date: 22 January 1998 (22.01.98)	
(22) International Filing Date: 15 July 1997 (15.07.97)		(74) Agents: BASTIAN, Kevin, L. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111 (US).	
(30) Priority Data: 08/680,395 15 July 1996 (15.07.96) US 08/731,499 16 October 1996 (16.10.96) US 08/785,532 17 January 1997 (17.01.97) US		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612 (US).		Published With international search report.	
(72) Inventors; and (75) Inventors/Applicants (for US only): GRAY, Joe, W. [US/US]; 1921 11th Avenue, San Francisco, CA 94116 (US). COLLINS, Colin, Conrad [US/US]; 333 Mountain View Avenue, San Rafael, CA 94901 (US). HWANG, Soo-In [US/US]; 189 Fairlawn Drive, Berkeley, CA 94708 (US). GODFREY, Tony [GB/US]; 699 Teresita Boulevard, San Francisco, CA 94127 (US). KOWBEL, David [US/US]; 6009 Auburn Avenue, Oakland, CA 94618 (US). ROMMENS, Johanna [CA/CA]; 717 Bay Street #1501, Toronto, Ontario M5G 2J9 (CA).			
(54) Title: GENES FROM 20q13 AMPLICON AND THEIR USES			
(57) Abstract			
<p>The present invention relates to cDNA sequences from a region of amplification on chromosome 20 associated with disease. The sequences can be used in hybridization methods for the identification of chromosomal abnormalities associated with various diseases. The sequences can also be used for treatment of diseases.</p>			

BEST AVAILABLE COPY

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## GENES FROM 20q13 AMPLICON AND THEIR USES

5

### CROSS-REFERENCE TO RELATED APPLICATIONS

10

This application is a continuation-in-part of USSN 08/785,532, filed on January 17, 1997, USSN 08/731,499 filed on October 16, 1996 and USSN 08/680,395 filed on July 15, 1996, which is related to copending U.S. Patent Application, USSN 08/546,130, filed October 20, 1995, each of which is incorporated herein by reference for all purposes.

15

### BACKGROUND OF THE INVENTION

This invention pertains to the field of cytogenetics. More particularly this invention pertains to the identification of genes in a region of amplification at about 20q13 in various cancers. The genes disclosed here can be used as probes specific for the 20q13 amplicon as well as for treatment of various cancers.

20

Chromosome abnormalities are often associated with genetic disorders, degenerative diseases, and cancer. In particular, the deletion or multiplication of copies of whole chromosomes or chromosomal segments, and higher level amplifications of specific regions of the genome are common occurrences in cancer. See, for example Smith, *et al.*, *Breast Cancer Res. Treat.*, 18: Suppl. 1: 5-14 (1991), van de Vijer & Nusse, *Biochim. Biophys. Acta*, 1072: 33-50 (1991), Sato, *et al.*, *Cancer. Res.*, 50: 7184-7189 (1990). In fact, the amplification and deletion of DNA sequences containing proto-oncogenes and tumor-suppressor genes, respectively, are frequently characteristic of tumorigenesis. Dutrillaux, *et al.*, *Cancer Genet. Cytogenet.*, 49: 203-217 (1990). Clearly, the identification of amplified and deleted regions and the cloning of the genes involved is crucial both to the study of tumorigenesis and to the development of cancer diagnostics.

25

30

The detection of amplified or deleted chromosomal regions has traditionally been done by cytogenetics. Because of the complex packing of DNA into the chromosomes, resolution of cytogenetic techniques has been limited to regions larger than about 10 Mb; approximately the width of a band in Giemsa-stained chromosomes. In complex karyotypes with multiple translocations and other genetic changes, traditional cytogenetic analysis is of little utility because karyotype information is lacking or cannot

be interpreted. Teyssier, J.R., *Cancer Genet. Cytogenet.*, 37: 103 (1989). Furthermore, conventional cytogenetic banding analysis is time consuming, labor intensive, and frequently difficult or impossible.

More recently, cloned probes have been used to assess the amount of a given DNA sequence in a chromosome by Southern blotting. This method is effective even if the genome is heavily rearranged so as to eliminate useful karyotype information. However, Southern blotting only gives a rough estimate of the copy number of a DNA sequence, and does not give any information about the localization of that sequence within the chromosome.

Comparative genomic hybridization (CGH) is a more recent approach to identify the presence and localization of amplified/deleted sequences. See Kallioniemi, et al., *Science*, 258: 818 (1992). CGH, like Southern blotting, reveals amplifications and deletions irrespective of genome rearrangement. Additionally, CGH provides a more quantitative estimate of copy number than Souther blotting, and moreover also provides information of the localization of the amplified or deleted sequence in the normal chromosome.

Using CGH, the chromosomal 20q13 region has been identified as a region that is frequently amplified in cancers (see, e.g. Kallioniemi et al., *Genomics*, 20: 125-128 (1994)). Initial analysis of this region in breast cancer cell lines identified a region approximately 2 Mb on chromosome 20 that is consistently amplified.

#### SUMMARY OF THE INVENTION

The present invention relates to the identification of a narrow region (about 600 kb) within a 2 Mb amplicon located at about chromosome 20q13 (more precisely at 20q13.2) that is consistently amplified in primary tumors. In addition, this invention provides cDNA sequences from a number of genes which map to this region. These sequences are useful as probes or as probe targets for monitoring the relative copy number of corresponding sequences from a biological sample such as a tumor cell. Also provided is a contig (a series of clones that contiguously spans this amplicon) which can be used to prepare probes specific for the amplicon. The probes can be used to detect chromosomal abnormalities at 20q13.

Thus, in one embodiment, this invention provides a method of detecting a chromosome abnormality (e.g., an amplification or a deletion) at about position FLpter

0.825 on human chromosome 20 (20q13.2). The method involves contacting a chromosome sample from a patient with a composition consisting essentially of one or more labeled nucleic acid probes each of which binds selectively to a target polynucleotide sequence at about position FLpter 0.825 on human chromosome 20 under conditions in which the probe forms a stable hybridization complex with the target sequence; and detecting the hybridization complex. The step of detecting the hybridization complex can involve determining the copy number of the target sequence. The probe preferably comprises a nucleic acid that specifically hybridizes under stringent conditions to a nucleic acid selected from the nucleic acids disclosed here. Even more preferably, the probe comprises a subsequence selected from sequences set forth in SEQ. ID. Nos. 1-10 and 12. The probe is preferably labeled, and is more preferably labeled with digoxigenin or biotin. In one embodiment, the hybridization complex is detected in interphase nuclei in the sample. Detection is preferably carried out by detecting a fluorescent label (e.g., FITC, fluorescein, or Texas Red). The method can further involve contacting the sample with a reference probe which binds selectively to a chromosome 20 centromere.

This invention also provides for two new genes, ZABC1 and 1b1, in the 20q13.2 region that are both amplified and overexpressed in a variety of cancers. ZABC1 is a putative zinc finger protein. Zinc finger proteins are found in a variety of transcription factors, and amplification or overexpression of transcription factors typically results in cellular mis-regulation. ZABC1 and 1b1 thus appear to play an important role in the etiology of a number of cancers.

This invention provides for a new human cyclophilin nucleic acid (SEQ ID NO 13). Cyclophilin nucleic acids have been implicated in a variety of cellular processes, including signal transduction.

This invention also provides for proteins encoded by nucleic acid sequences in the 20q13 amplicon (SEQ. ID. Nos: 1-10 and 12-13) and subsequences, more preferably subsequences of at least 10 amino acids, preferably of at least 20 amino acids and most preferably of at least 30 amino acids in length. Particularly preferred subsequences are epitopes specific to the 20q13 proteins, more preferably epitopes specific to the ZABC1 and 1b1 proteins. Such proteins include, but are not limited to isolated polypeptides comprising at least 20 amino acids from a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12-13 or from the polypeptide of SEQ. ID. No. 11

wherein the polypeptide, when presented as an immunogen, elicits the production of an antibody which specifically binds to a polypeptide selected from the group consisting of a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12-13 or from the polypeptide of SEQ. ID. No. 11, where the polypeptide does not bind to antisera raised against a polypeptide selected from the group consisting of a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12-13 or from the polypeptide of SEQ. ID. No. 11. In preferred embodiments, the polypeptides of the invention hybridize to antisera raised against a polypeptide encoded by those encoded by SEQ ID NOs. 1-13, where the antisera has been immunosorbed with the most structurally related previously known polypeptide. For example, a polypeptide of the invention binds to antisera raised against a polypeptide encoded by SEQ ID NO. 13, wherein the antisera has been immunosorbed with a rat or mouse cyclophilin polypeptide (Rat cyclophilin nucleic acids are known; see, GenBank™ under accession No. M19533; Mouse cyclophilin nucleic acids are known; see, GenBank™ under accession No. 50620. cDNAs from the mouse and rat cyclophilin cDNAs are about 85% identical to SEQ ID NO. 13).

In another embodiment, the method can involve detecting a polypeptide (protein) encoded by a nucleic acid (ORF) in the 20q13 amplicon. The method may include any of a number of well known protein detection methods including, but not limited to, the protein assays disclosed herein.

This invention also provides cDNA sequences from genes in the amplicon (SEQ. ID. Nos. 1-10 and 12-13). The nucleic acid sequences can be used in therapeutic applications according to known methods for modulating the expression of the endogenous gene or the activity of the gene product. Examples of therapeutic approaches include antisense inhibition of gene expression, gene therapy, monoclonal antibodies that specifically bind the gene products, and the like. The genes can also be used for recombinant expression of the gene products *in vitro*.

This invention also provides for proteins (e.g., SEQ. ID. No. 11) encoded by the cDNA sequences from genes in the amplicon (e.g., SEQ. ID. Nos. 1-10 and 12-13). Where the amplified nucleic acids include cDNA which are expressed, detection and/or quantification of the protein expression product can be used to identify the presence

or absence or quantify the amplification level of the amplicon or of abnormal protein products produced by the amplicon.

The probes disclosed here can be used in kits for the detection of a chromosomal abnormality at about position FLpter 0.825 on human chromosome 20. The 5 kits include a compartment which contains a labeled nucleic acid probe which binds selectively to a target polynucleotide sequence at about FLpter 0.825 on human chromosome 20. The probe preferably includes at least one nucleic acid that specifically hybridizes under stringent conditions to a nucleic acid selected from the nucleic acids disclosed here. Even more preferably, the probes comprise one or more nucleic acids 10 selected from the nucleic acids disclosed here. In a preferred embodiment, the probes are labeled with digoxigenin or biotin. The kit may further include a reference probe specific to a sequence in the centromere of chromosome 20.

#### Definitions

A "nucleic acid sample" as used herein refers to a sample comprising DNA 15 in a form suitable for hybridization to a probes of the invention. The nucleic acid may be total genomic DNA, total mRNA, genomic DNA or mRNA from particular chromosomes, or selected sequences (e.g. particular promoters, genes, amplification or restriction fragments, cDNA, etc.) within particular amplicons disclosed here. The nucleic acid sample may be extracted from particular cells or tissues. The tissue sample from which 20 the nucleic acid sample is prepared is typically taken from a patient suspected of having the disease associated with the amplification being detected. In some cases, the nucleic acids may be amplified using standard techniques such as PCR, prior to the hybridization. The sample may be isolated nucleic acids immobilized on a solid surface (e.g., nitrocellulose) for use in Southern or dot blot hybridizations and the like. The sample 25 may also be prepared such that individual nucleic acids remain substantially intact and comprises interphase nuclei prepared according to standard techniques. A "nucleic acid sample" as used herein may also refer to a substantially intact condensed chromosome (e.g. a metaphase chromosome). Such a condensed chromosome is suitable for use as a hybridization target in *in situ* hybridization techniques (e.g. FISH). The particular usage 30 of the term "nucleic acid sample" (whether as extracted nucleic acid or intact metaphase chromosome) will be readily apparent to one of skill in the art from the context in which the term is used. For instance, the nucleic acid sample can be a tissue or cell sample

prepared for standard *in situ* hybridization methods described below. The sample is prepared such that individual chromosomes remain substantially intact and typically comprises metaphase spreads or interphase nuclei prepared according to standard techniques.

5 A "chromosome sample" as used herein refers to a tissue or cell sample prepared for standard *in situ* hybridization methods described below. The sample is prepared such that individual chromosomes remain substantially intact and typically comprises metaphase spreads or interphase nuclei prepared according to standard techniques.

10 "Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

15 An "isolated" polynucleotide is a polynucleotide which is substantially separated from other contaminants that naturally accompany it, e.g., protein, lipids, and other polynucleotide sequences. The term embraces polynucleotide sequences which have been removed or purified from their naturally-occurring environment or clone library, and include recombinant or cloned DNA isolates and chemically synthesized analogues or analogues biologically synthesized by heterologous systems.

20 "Subsequence" refers to a sequence of nucleic acids that comprise a part of a longer sequence of nucleic acids.

25 A "probe" or a "nucleic acid probe", as used herein, is defined to be a collection of one or more nucleic acid fragments whose hybridization to a target can be detected. The probe may be unlabeled or labeled as described below so that its binding to the target can be detected. The probe is produced from a source of nucleic acids from one or more particular (preselected) portions of the genome, for example one or more clones, an isolated whole chromosome or chromosome fragment, or a collection of polymerase chain reaction (PCR) amplification products. The probes of the present invention are produced from nucleic acids found in the 20q13 amplicon as described herein. The probe may be processed in some manner, for example, by blocking or removal of repetitive nucleic acids or enrichment with unique nucleic acids. Thus the word "probe" may be used herein to refer not only to the detectable nucleic acids, but to the detectable nucleic

acids in the form in which they are applied to the target, for example, with the blocking nucleic acids, *etc.* The blocking nucleic acid may also be referred to separately. What "probe" refers to specifically is clear from the context in which the word is used.

The probe may also be isolated nucleic acids immobilized on a solid surface (e.g., nitrocellulose). In some embodiments, the probe may be a member of an array of nucleic acids as described, for instance, in WO 96/17958. Techniques capable of producing high density arrays can also be used for this purpose (see, e.g., Fodor *et al.* *Science* 267:773 (1991) and U.S. Patent No. 5,143,854).

"Hybridizing" refers the binding of two single stranded nucleic acids via complementary base pairing.

"Bind(s) substantially" or "binds specifically" or "binds selectively" or "hybridizes specifically" refer to complementary hybridization between an oligonucleotide and a target sequence and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence. These terms also refer to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (e.g., total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. "Stringent hybridization" and "Stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as CGH, FISH, Southern and northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* part I chapter 2 "overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5° C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the  $T_m$  for a particular probe.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formalin with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of stringent wash conditions is a .2x SSC wash at 65°C for 15 minutes (see, Sambrook, *supra* for a description of SSC buffer). Often, the high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., about 100 nucleotides or more, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

One of skill will recognize that the precise sequence of the particular probes described herein can be modified to a certain degree to produce probes that are "substantially identical" to the disclosed probes, but retain the ability to bind substantially to the target sequences. Such modifications are specifically covered by reference to the individual probes herein. The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 90% sequence identity, more preferably at least 95%, compared to a reference sequence using the methods described below using standard parameters.

Two nucleic acid sequences are said to be "identical" if the sequence of nucleotides in the two sequences is the same when aligned for maximum correspondence as described below. The term "complementary to" is used herein to mean that the complementary sequence is identical to all or a portion of a reference polynucleotide sequence. Nucleic acids which do not hybridize to complementary versions of each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

Sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two sequences over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a segment of at least about 20 contiguous positions, usually about 50

to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444 (1988), by computerized implementations of these algorithms.

"Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to the same nucleic acid sequence under stringent conditions.

"Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of "conservatively modified variations." Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques. Accordingly,

each "silent variation" of a nucleic acid which encodes a polypeptide is implicit in each described sequence. Furthermore, one of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. The following six groups each contain amino acids that are conservative substitutions for one another:

- 10            1) Alanine (A), Serine (S), Threonine (T);  
              2) Aspartic acid (D), Glutamic acid (E);  
              3) Asparagine (N), Glutamine (Q);  
              4) Arginine (R), Lysine (K);  
              5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and  
15            6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The term "20q13 amplicon protein" is used herein to refer to proteins encoded by ORFs in the 20q13 amplicon disclosed herein. Assays that detect 20q13 amplicon proteins are intended to detect the level of endogenous (native) 20q13 amplicon proteins present in subject biological sample. However, exogenous 20q13 amplicon proteins (from a source extrinsic to the biological sample) may be added to various assays to provide a label or to compete with the native 20q13 amplicon protein in binding to an anti-20q13 amplicon protein antibody. One of skill will appreciate that a 20q13 amplicon protein mimetic may be used in place of exogenous 20q13 protein in this context. A "20q13 protein", as used herein, refers to a molecule that bears one or more 20q13 amplicon protein epitopes such that it is specifically bound by an antibody that specifically binds a native 20q13 amplicon protein.

As used herein, an "antibody" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or

lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

The basic immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain ( $V_L$ ) and variable heavy chain ( $V_H$ ) refer to these light and heavy chains respectively.

Antibodies may exist as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce  $F(ab')_2$ , a dimer of Fab which itself is a light chain joined to  $V_H-C_H1$  by a disulfide bond. The  $F(ab')_2$  may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the  $F(ab')_2$  dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, *Fundamental Immunology*, W.E. Paul, ed., Raven Press, N.Y. (1993) for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA methodology.

Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized *de novo* using recombinant DNA methodologies.

The phrase "specifically binds to a protein" or "specifically immunoreactive with", when referring to an antibody refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies can be raised to the a 20q13 amplicon protein that bind the 20q13 amplicon protein and not to any other proteins present in a biological sample. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a

particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

5

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1(A) shows disease-free survival of 129 breast cancer patients according to the level of 20q13 amplification. Patients with tumors having high level 20q13 amplification have a shorter disease-free survival ( $p=0.04$  by Mantel-Cox test) compared to those having no or low level amplification.

10 Figure 1(B) Shows the same disease-free survival difference of Figure 1(A) in the sub-group of 79 axillary node-negative patients ( $p=0.0022$  by Mantel-Cox test).

15 Figure 2 shows a comparison of 20q13 amplification detected by FISH in a primary breast carcinoma and its metastasis from a 29-year patient. A low level amplification of 20q13 (20q13 compared to 20p reference probe) was found in the primary tumor. The metastasis, which appeared 8 months after mastectomy, shows a high level amplification of the chromosome 20q13 region. The overall copy number of chromosome 20 (20p reference probe) remained unchanged. Each data point represents gene copy numbers in individual tumor cells analyzed.

20 Figure 3 shows a graphical representation of the molecular cytogenetic mapping and subsequent cloning of the 20q13.2 amplicon. Genetic distance is indicated in centiMorgans (cM). The thick black bar represents the region of highest level amplification in the breast cancer cell line BT474 and covers a region of about 1.5 Mb. P1 and BAC clones are represented as short horizontal lines and YAC clones as heavier horizontal lines. Not all YAC and P1 clones are shown. YACs 957f3, 782c9, 931h12, and 902 are truncated. Sequence tagged sites (STSs) appear as thin vertical lines and open circles indicate that a given YAC has been tested for and is positive for a given STS. Not all STSs have been tested on all YACs. The interval from which more than 100 exons have been trapped is represented as a filled box. The 600 kb interval spanning the region 25 of highest amplification level in primary tumors is represented by the filled black box (labeled Sequence). The lower part of the figure shows the levels of amplification in two

30

primary tumors that further narrow the region of highest amplification to within about 600 kb.

Figure 4 provides a higher resolution map of the amplicon core as defined in primary tumors.

5 Figure 5 shows the map location of 15 genes in the amplicon.

Figure 6 shows a sequence alignment between Rat cyclophilin and SEQ ID  
13.

10 Figure 7 is a physical map of the 20q13 amplicon. Row A of the figure shows the position of STSs used to assemble the map. Row B shows the position of YACs. Row C shows the position of P1 and BAC clones. Row D shows the position of trapped exons, direct selected cDNAs, ESTs, and genes. Row E shows results of hybridization analysis of various primary tumors and cell lines using P1 or BAC clones as noted there. A solid black circle indicates high amplification, a shaded circle indicates intermediate amplification and an open circle indicates no amplification as detected by each of the clones.

#### 15 DETAILED DESCRIPTION

This invention provides a number of cDNA sequences which can be used as probes for the detection of chromosomal abnormalities at 20q13. Studies using comparative genomic hybridization (CGH) have shown that a region at chromosome 20q13 is increased in copy number frequently in cancers of the breast (~30%), ovary (~ 15%), bladder (~30%), head and neck (~75%) and colon (~80%). This suggests the presence of one or more genes that contribute to the progression of several solid tumors are located at 20q13.

Gene amplification is one mechanism by which dominantly acting oncogenes are overexpressed, allowing tumors to acquire novel growth characteristics and/or resistance to chemotherapeutic agents. Loci implicated in human breast cancer progression and amplified in 10-25% of primary breast carcinomas include the erbB-2 locus (Lupu *et al.*, *Breast Cancer Res. Treat.*, 27: 83 (1993), Slamon *et al.* *Science*, 235: 177-182 (1987), Heiskanen *et al.* *Biotechniques*, 17: 928 (1994)) at 17q12, cyclin-D (Mahadevan *et al.*, *Science*, 255: 1253-1255 (1993), Gillett *et al.*, *Canc. Res.*, 54: 1812 (1994)) at 11q13 and MYC (Gaffey *et al.*, *Mod. Pathol.*, 6: 654 (1993)) at 8q34.

Pangenomic surveys using comparative genomic hybridization (CGH) recently identified about 20 novel regions of increased copy number in breast cancer (Kallioniemi *et al.*, *Genomics*, 20: 125-128 (1994)). One of these loci, band 20q13, was amplified in 18% of primary tumors and 40% of cell lines (Kallioniemi *et al.*, *Genomics*, 20: 125-128 (1994)). More recently, this same region was found amplified in 15% of ovarian, 80% of bladder and 80% of colorectal tumors. The resolution of CGH is limited to 5-10 Mb. Thus, FISH was performed using locus specific probes to confirm the CGH data and precisely map the region of amplification.

The 20q13 region has been analyzed in breast cancer at the molecular level and a region, approximately 600 kb wide, that is consistently amplified was identified, as described herein. Moreover, as shown herein, the importance of this amplification in breast cancer is indicated by the strong association between amplification and decreased patient survival and increased tumor proliferation (specifically, increased fraction of cells in S-phase).

In particular, as explained in detail in Example 1, high-level 20q13 amplification was associated ( $p=0.0022$ ) with poor disease free survival in node-negative patients, compared to cases with no or low-level amplification (Figure 1). Survival of patients with moderately amplified tumors did not differ significantly from those without amplification. Without being bound to a particular theory, it is suggested that an explanation for this observation may be that low level amplification precedes high level amplification. In this regard, it may be significant that one patient developed a local metastasis with high-level 20q13.2 amplification 8 month after resection of a primary tumor with low level amplification (Figure 2).

The 20q13 amplification was associated with high histologic grade of the tumors. This correlation was seen in both moderately and highly amplified tumors. There was also a correlation ( $p=0.0085$ ) between high level amplification of a region complementary to a particular probe, RMC20C001 (Tanner *et al.*, *Cancer Res.*, 54: 4257-4260 (1994)), and cell proliferation, measured by the fraction of cells in S-phase. This finding is important because it identifies a phenotype that can be scored in functional assays, without knowing the mechanism underlying the increased S-phase fraction. The 20q13 amplification did not correlate with the age of the patient, primary tumor size, axillary nodal or steroid hormone-receptor status.

This work localized the 20q13.2 amplicon to an interval of approximately 2 Mb. Furthermore, it suggests that high-level amplification, found in 7% of the tumors, confers an aggressive phenotype on the tumor, adversely affecting clinical outcome. Low level amplification (22% of primary tumors) was associated with pathological features typical of aggressive tumors (high histologic grade, aneuploidy and cell proliferation) but not patient prognosis.

In addition, it is shown herein that the 20q13 amplicon (more precisely the 20q13.2 amplicon) is one of three separate co-amplified loci on human chromosome 20 that are packaged together throughout the genomes of some primary tumors and breast cancer cell lines. No known oncogenes map in the 20q13.2 amplicon.

#### Identification of 20q13 Amplicon Probes.

Initially, a paucity of available molecular cytogenetic probes dictated that FISH probes be generated by the random selection of cosmids from a chromosome 20 specific library, LA20NC01, and then mapped them to chromosome 20 by digital imaging microscopy. Approximately 46 cosmids, spanning the 70 Mb chromosome, were isolated for which fractional length measurements (FL<sub>pter</sub>) and band assignments were obtained. Twenty six of the cosmids were used to assay copy number in the breast cancer cell line BT474 by interphase FISH analysis. Copy number was determined by counting hybridization signals in interphase nuclei. This analysis revealed that cosmid RMC20C001 (FL<sub>pter</sub>, 0.824; 20q13.2), described by Stokke *et al.*, *Genomics*, 26: 134-137 (1995), defined the highest-level amplification (~60 copies/cell) in BT474 cells (Tanner *et al.*, *Cancer Res.*, 54: 4257-4260 (1994)).

P1 clones containing genetically mapped sequences were selected from 20q13.2 and used as FISH probes to confirm and further define the region of amplification. Other P1 clones were selected for candidate oncogenes broadly localized to the 20q13.2 region (FL<sub>pter</sub>, 0.81-0.84). These were selected from the DuPont P1 library (Shepherd, *et al.*, *Proc. Natl. Acad. Sci. USA*, 92: 2629 (1994), available commercially from Genome Systems), by PCR (Saiki *et al.*, *Science*, 230: 1350 (1985)) using primer pairs developed in the 3' untranslated region of each candidate gene. Gene specific P1 clones were obtained for, protein tyrosine phosphatase (PTPN1, FL<sub>pter</sub> 0.78), melanocortin 3 receptor (MC3R, FL<sub>pter</sub> 0.81), phosphoenolpyruvate carboxy kinase (PCK1, FL<sub>pter</sub> 0.85), zinc finger protein 8 (ZNF8, FL<sub>pter</sub> 0.93), guanine

nucleotide-binding protein (GNAS 1, Flpter .873), src-oncogene (SRC, Flpter 0.669), topoisomerase 1 (TOP1, Flpter 0.675), the bcl-2 related gene bcl-x (Flpter 0.526) and the transcription factor E2F-1 (FLpter 0.541). Each clone was mapped by digital imaging microscopy and assigned Flpter values. Five of these genes (SRC, TOPO1, GNAS1, 5 E2F-1 and BC1-x) were excluded as candidate oncogenes in the amplicon because they mapped well outside the critical region at Flpter 0.81-0.84. Three genes (PTPNR1, PCK-1 and MC3R) localized close enough to the critical region to warrant further investigation.

Interphase FISH on 14 breast cancer cell lines and 36 primary tumors using 10 24 cosmid and 3 gene specific P1 (PTPNR1, PCK-1 and MC3R) probes found high level amplification in 35% (5/14) of breast cancer cell lines and 8% (3/36) of primary tumors with one or more probe. The region with the highest copy number in 4/5 of the cell lines and 3/3 primary tumors was defined by the cosmid RMC20C001. This indicated that 15 PTPNR1, PCK1 and MC3R could also be excluded as candidates for oncogenes in the amplicon and, moreover, narrowed the critical region from 10 Mb to 1.5-2.0 Mb (see, Tanner *et al.*, *Cancer Res.*, 54: 4257-4260 (1994)).

Because probe RMC20C001 detected high-level (3 to 10-fold) 20q13.2 amplification in 35% of cell lines and 8% of primary tumors it was used to (1) define the prevalence of amplification in an expanded tumor population, (2) assess the frequency and 20 level of amplification in these tumors, (3) evaluate the association of the 20q13.2 amplicon with pathological and biological features, (4) determine if a relationship exists between 20q13 amplification and clinical outcome and (5) assess 20q13 amplification in metastatic breast tumors.

As detailed in Example 1, fluorescent *in situ* hybridization (FISH) with 25 RMC20C001 was used to assess 20q13.2 amplification in 132 primary and 11 recurrent breast tumors. The absolute copy number (mean number of hybridization signals per cell) and the level of amplification (mean number of signals relative to the p-arm reference probe) were determined. Two types of amplification were found: Low level amplification (1.5-3 fold with FISH signals dispersed throughout the tumor nuclei) and high level 30 amplification (>3 fold with tightly clustered FISH signals). Low level 20q13.2 amplification was found in 29 of the 132 primary tumors (22%), whereas nine cases (6.8%) showed high level amplification.

RMC20C001 and four flanking P1 probes (MC3R, PCK, RMC20C026, and RMC20C030) were used to study the extent of DNA amplification in highly amplified tumors. Only RMC20C001 was consistently amplified in all tumors. This finding confirmed that the region of common amplification is within a 2 Mb interval flanked by 5 but not including PCK-1 and MC3R.

A physical map was assembled to further localize the minimum common region of amplification and to isolate the postulated oncogene(s). The DuPont P1 library (Shepherd *et al. Proc. Natl. Acad. Sci. USA*, 91: 2629 (1994) was screened for STSs likely to map in band 20q13.2. P1 clones at the loci D20S102, D20S100, D20S120, 10 D20S183, D20S480, D20S211 were isolated, and FISH localized each to 20q13.2. Interphase FISH analysis was then performed in the breast cancer cell line BT474 to assess the amplification level at each locus. The loci 15 D20S100-D20S120-D20S183-D20S480-D20S211 were highly amplified in the BT474 cell line, whereas D20S102 detected only low level amplification. Therefore, 5 STSs, spanning 5 cM, were localized within the 20q13.2 amplicon and were utilized to screen the CEPH megaYAC library.

CEPH megaYAC library screening and computer searches of public databases revealed D20S120-D20S183-D20S480-D20S211 to be linked on each of three megaYAC clones y820f5, 773h10, and 931h6 (Figure 3). Moreover, screening the CEPH megaYAC library with STSs generated from the ends of cosmids RMC20C001, 20 RMC20C30 and RMC20C028 localized RMC20C001 to each of the same three YAC clones. It was estimated, based on the size of the smallest of these YAC clones, that D20S120-D20S 183-RMC20C001-D20S480-D20S211 map into an interval of less than 1.1 Mb. D20S100 was localized 300 kb distal to D20S120 by interphase FISH and to 25 YAC901b12 by STS mapping. The combined STS data made it possible to construct a 12 member YAC contig which spans roughly 4 Mb encompassing the 1.5 Mb amplicon and containing the loci RMC20C030-PCK1-RMC20C001-MC3R-RMC20CO26. Each YAC was mapped by FISH to confirm localization to 20q13.2 and to check for chimerism. Five 30 clonal isolates of each YAC were sized by pulsed field gel electrophoresis (PFGE). None of the YACs are chimeric, however, several are highly unstable.

The YAC contig served as a framework from which to construct a 2 MbP1 contig spanning the 20Q13 amplicon. P1 clones offered numerous advantages over YAC

clones including (1) stability, (2) a chimeric frequency of less than 1%, (3) DNA isolation by standard miniprep procedures, (4) they make ideal FISH probes, (5) the ends can be sequenced directly, (6) engineered  $\gamma\delta$  transposons carrying bidirectional primer binding sites can be integrated at any position in the cloned DNA (Strathmann *et al.*, *Proc. Natl. Acad. Sci. USA*, 88: 1247 (1991)) (7) P1 clones are the templates for sequencing the human and Drosophila genomes at the LBNL HGC (Palazzolo *et al.* *DOE Human Genome Program, Contractor-Grantee Workshop IV, Santa Fe, New Mexico, November 13-17 1994*).

About 90 P1 clones were isolated by screening the DuPont P1 library either by PCR or filter hybridization. For PCR based screening, more than 22 novel STSs were created by two methods. In the first method, the ends of P1 clones localized to the amplicon were sequenced, STSs developed, and the P1 library screened for walking clones. In the second approach inter-Alu PCR (Nelson *et al.*, 86: 6686-6690 (1989)) was performed on YACs spanning the amplicon and the products cloned and sequenced for STS creation. In the filter based hybridization scheme P1 clones were obtained by performing inter-Alu PCR on YACs spanning the amplicon, radio-labeling the products and hybridizing these against filters containing a gridded array of the P1 library. Finally, to close gaps a human genomic bacterial artificial chromosome (BAC) library (Shizuya *et al.* *Proc. Natl. Acad. Sci. USA*, 89: 8794 (1992), commercially available from Research Genetics, Huntsville, Alabama, USA) was screened by PCR. These methods combined to produce more than 100 P1 and BAC clones were localized to 20q13.2 by FISH. STS content mapping, fingerprinting, and free-chromatin fish (Heiskanen *et al.*, *BioTechniques*, 17: 928 (1994)) were used to construct the 2 Mb contig shown in Figure 3 as well as in Figure 4. Figure 7 is a second physical map of the region showing a minimum tiling contig.

#### Fine Mapping the 20q13.2 Amplicon in BT474

Clones from the 2 Mb P1 contig were used with FISH to map the level of amplification at 20q13.2 in the breast cancer cell line BT474. 35 P1 probes distributed at regular intervals along the contig were used. The resulting data indicated that the region of *highest copy number increase* in BT474 occurs between D20S100 and D20S211, an interval of approximately 1.5 Mb. P1 FISH probes, in this interval, detect an average of 50 signals per interphase nuclei in BT474, while no, or only low level amplification, was

detected with the P1 clones outside this region. Thus, both the proximal and distal boundaries of the amplicon were cloned.

Fine Mapping the 20q13.2 Amplicon in Primary Tumors.

5 Fine mapping the amplicon in primary tumors revealed the *minimum common region of high amplification* (MCA) that is of pathobiological significance. This process is analogous to screening for informative meiosis in the narrowing of genetic intervals encoding heritable disease genes. Analysis of 132 primary tumors revealed thirty-eight primary tumors that are amplified at the RMC20C001 locus. Nine of these tumors have high level amplification at the RMC20C001 locus and were further analyzed  
10 by interphase FISH with 8 P1s that span the  $\approx$ 2 Mb contig. The minimum common region of amplification (MCA) was mapped to a  $\approx$ 600 kb interval flanked by P1 clones #3 and #12 with the highest level of amplification detected by P1 clone #38 corresponding to RMC20C001 (Figures 3 and 7).

15 The P1 and BAC clones spanning the 600 kb interval of the 20q13 amplicon are listed in Tables 1 and 2 which provide a cross-reference to the DuPont P1 library described by Shepherd, *et al.*, Proc. Natl. Acad. Sci. USA, 92: 2629 (1994). These P1 and BAC probes are available commercially from Genetic Systems, and Research Genetics, respectively).

**Table 1.** Cross reference to probes of the DuPont PI library (Shepherd, et al., Proc. Natl. Acad. Sci. USA, 92:2629 (1994) which is commercially available from Genomic Systems, St. Louis, Missouri, USA). PCR primers are illustrated for amplification of Sequence tag sites for each clone. In addition, PCR conditions (Mg concentration and annealing temperature), as well as PCR product size, is provided. Size: PCR product Size; mM MgCl: Mg concentration; Ann.: Annealing temperature; P1: P1 probe ID number; PC: DuPont Library Plate Coordinates; SCA: DuPont Library Single Clone address; SEQ-forward and SEQ-backward: forward and backward PCR primers, respectively; SEQ ID NO:-forward and SEQ ID NO:-backward: Sequence Listing SEQ ID NO: for forward and backward primers, respectively.

PRIMER NAME	SIZE (bp)	mM MgCl	Ann.	P1	PC	SCA	SEQ-forward	SEQ-backward	SEQ ID NO:- NO:forward	SEQ ID NO:- NO:backward
352_32	136	1.5	52	20	103-5	1228e	TTCGCATTTGTTATCAGGTAGCTG	TGGAGCGAGAGGGGATTGTTGTTG	1	2
388_13 F1/B1	201	1.5	52	17	6986	821	AATCCCCCTCAAAACCTTGCTGCTAC	TGGAGCCCTGAACTCTCTGCAATC	3	4
				19	9874	1167f			-	-
D20S183	270	3	48	30		1246f	CCGGGATACCCOACATG	TGACACATAAAACAGCCAGC	5	6
				40	24h1	276h			-	-
D20S211 4	135	1.5	52	29	119f4	1418f	TTGGAAATCAAATGGAGCAAAA	AGCTTACCCAAATGGGGTCC	7	8
D20S480	300	3	55	68	100812	1199d2	GTCGTAACACCCAATAAATGG	AAAGCAAAATAAAACCAATAAACTCA	9	10
				41	86e1	1020c			-	-
				42	103d9	1232d			-	-
				67	91b2	1081b9			-	-
9X SP6 hmF/hmB	165	1.5	55	7	31d11	370d	CAAQATCTGACCCCCGTCAATC	GAATCTTCAGGAAAGAGATCAGTG	11	12
				9	3519	416f			-	-
11S-17 F2/B4	146	3	58	11	41b1	480b	GCCATGTAACCCACCTGAAAAATC	TCAGAACACCCCCTGAGAAATTAG	13	14
12T-T7 F2/B1	153	3	58	12	42c2	493c	CCTAAAACCTTGATGCTTAATCTA	GTCTCACAAAGGCAAGATGTOG	15	16
28T F1/B1	219	1.5	52	74		888f2	TTTGTGTATGTTAGGCCATC	CTTCACATCTCATCTCATGAGG	17	18
				2	12c6	137c			-	-
				25	118e11	1413c			-	-
				26	118e11	1413c			-	-
28S F1/B3	214	1.5	55	28	118g11	1413g	GCTTGTGTTAGTGTACTAGGG	CACTCTGGTAATGACCTTTGTC	19	20
				25	118e11	1413c			-	-
				27	118g11	1413g			-	-
				10	36n0	429f			-	-
69S	100	3	55	69		412b5	CCTACACCATCCAACTTGA	CCAGATGTTATGTTGCTACGGAAAC	21	22
HSCOFH032 F/B	129	1.5	55	3	23e1	264c			-	-
				12-e11	142e	TCTCAAAACCTGTCACCTCTG	CTACTGTGGGGAGAATGG	23	24	
				18	77e10	921e			-	-

PRIMER NAME	SIZE (bp)	mM MgCl <sub>2</sub>	Am.	P1	PC	SCA	SEQ-forward	SEQ-backward	SEQ ID NO.: NO.: forward backward
60A1	191	1.5	58	36	11288	11398	TGTCTCTTCTCATCCATCCTAC	AAAGCCCTCCACTCACAGGATA	25 26
					39	346	401a	-	-
820F5A1T F1/B1	175	1.5	48	15	5367	630c	CCTCTTCAGTGTTCTCTTATGA	GGGAGGAGATTGTAGGC AAC	27 28
					16	5389	697b	-	-
103113377F31B3					185		1141D7		

**Table 2.** Cross reference to probes of the BAC library. Clone # refers to the clone number provided, e.g., in Figures 3, 4 and 7, while the plate coordinates are the plate coordinates in the Research Genetics BAC library. Size: PCR product Size; mM MgCl<sub>2</sub>: Mg concentration; Am.: Annealing temperature; BAC #: BAC probe ID number; SEQ-forward and SEQ-backward: forward and backward PCR primers, respectively; SEQ ID NO.:forward and SEQ ID NO.:backward. Sequence Listing SEQ ID NO.: for forward and backward primers, respectively.

PRIMER NAME	SIZE (bp)	mM MgCl <sub>2</sub>	Am.	BAC	BAC Plate Coordinates	SEQ-forward	SEQ-backward	SEQ ID NO.: forward	SEQ ID NO.: backward
18T F1/B1	156	3	62	99	L1 plate 146	AGCAAAGCAAAAGTGCGCAC	TGACATGGAGAAAACACACTTC	29	30
9S F1/B1	214	1.5	55	97	E8 plate 183	AGGTATTACCAATGTGTTGA	TCTACATCCCATTCTCTCTG	31	12
D20S480	300	3	55	95	H15 plate 140	ATGGTGAAACACCATAAAATGG	AAAGCAAAATAAACCCATAAACCTG	9	10
D20S211 1/2	135	1.5	52	103	A15 plate 188	TTGAAATCAATGGAGAAAAA	AGCTTATACCCAAATGTAATGCC	7	8
					102	A1 plate 46	-	-	-
11S-17 F2/B4	146	3	58	100	E4 plate 43	GCCATATGACCCACCTGAAAATTC	TCAGAACACCCCCGTGCAAGAATTAAAG	13	14
					101	J5 plate 118	-	-	-
CYP2A						87	J14 plate 96		
ET4211						104	C10 plate 754		
ET03.17						121	C10 plate 10		
D20S902						166	I4 plate 226		
180-R F1/B1						188	G21 plate 163		
164-1Fm/h2						197	N13 plate 309		

**cDNA sequences from the 20q13 amplicon.**

Exon trapping (see, e.g., Duyk *et al.*, *Proc. Natl. Acad. Sci. USA*, 87: 8995-8999 (1990) and Church *et al.*, *Nature Genetics*, 6: 98-105 (1994)) was performed on the P1 and BAC clones spanning the ≈600 kb minimum common region of amplification and has isolated more than 200 exons.

Analysis of the exons DNA sequence revealed a number of sequence similarities (85% to 96%) to partial CDNA sequences in the expressed sequence data base (dbest) and to a *S. cerevisiae* chromosome XIV open reading frame. Each P1 clone subjected to exon trapping has produced multiple exons consistent with at least a medium density of genes. Over 200 exons have been trapped and analyzed as well as 200 clones isolated by direct selection from a BT474 cDNA library. In addition a 0.6 Mb genomic interval spanning the minimal amplicon described below is being sequenced. Exon prediction and gene modeling are carried out with XGRAIL, SORFIND, and BLAST programs. Gene fragments identified by these approaches have been analyzed by RT-PCR, Northern and Southern blots. Fifteen unique genes were identified in this way (see, Table 3 and Figure 5).

In addition two other genes ZABC1 (SEQ. ID. 9 and 10) and 1b1 (SEQ. ID No. 12) were also shown to be overexpressed in a variety of different cancer cells.

Sequence information from various cDNA clones are provided below.

They are as follows:

3bf4 (SEQ. ID. No. 1) - 3kb transcript with sequence identity to a tyrosine kinase gene, termed A6, disclosed in Beeler *et al. Mol. Cell. Biol.* 14:982-988 (1994) and WO 95/19439. These references, however, do not disclose that the gene is located in the chromosome 20 amplicon.

1b11 (SEQ. ID. No. 2) - an approximately 3.5 kb transcript whose expression shows high correlation with the copy number of the amplicon. The sequence shows no homology with sequences in the databases searched.

cc49 (SEQ. ID. No. 3) - a 6-7 kb transcript which shows homology to C2H2 zinc finger genes.

cc43 (SEQ. ID. No. 4) - an approximately 4 kb transcript which is expressed in normal tissues, but whose expression in the breast cancer cell line has not been detected.

5           41.1 (SEQ. ID. No. 5) - shows homology to the homeobox T shirt gene in Drosophila.

GCAP (SEQ. ID. No. 6) - encodes a guanino cyclase activating protein which is involved in the biosynthesis of cyclic AMP. As explained in detail below, sequences from this gene can also be used for treatment of retinal degeneration.

1b4 (SEQ. ID. No. 7) - a serine threonine kinase.

10           20sa7 (SEQ. ID. No. 8) - a homolog of the rat gene, BEM-1.

In addition, the entire nucleotide sequence is provided for ZABC-1. ZABC-1 stands for zinc finger amplified in breast cancer. This gene maps to the core of the 20q13.2 amplicon and is overexpressed in primary tumors and breast cancer cell lines having 20q13.2 amplification. The genomic sequence (SEQ. ID. No. 9) includes roughly 2kb of the promoter region. SEQ ID. No. 10 provides the cDNA sequence derived open reading frame and SEQ ID. No. 11 provides the predicted protein sequence. Zinc finger containing genes are often transcription factors that function to modulate the expression of down stream genes. Several known oncogenes are in fact zinc finger containing genes.

20           This invention also provides the full length cDNA sequence for a cDNA designated 1b1 (SEQ. ID. No. 12) which is also overexpressed in numerous breast cancer cell lines and some primary tumors.

25           SEQ ID NO: 13 provides sequence from a genomic clone which is similar to known rat and mouse cyclophilin cDNAs. Rat Cyclophilin nucleic acids (e.g., cDNAs) are known; see, GenBank™ under accession No. M19533; Mouse Cyclophilin nucleic acids (e.g., cDNAs) are known; see, GenBank™ under accession No. 50620 (see, Figure 6). Accordingly, SEQ ID NO: 13 is a putative human cyclophilin gene. The sequence is also associated with amplified sequences from 20q13, and can be used as a probe or probe hybridization target to detect DNA amplification, or RNA overexpression of the corresponding gene.

**Table 3.** Gene fragments identified by exon trapping and analyzed by RT-PCR,

Northern and Southern blots.

Map #	Gene ID	Transcript Size (kb)	EST Identity (> 95 %)	Cloned Sequence (kb)	Protein Homologies	Map Location
5	1 20sa7	-	Yes	3	PTP	3, 18, 99
	2 1b11	3.5	No	1.5	novel	18, 3, 99, 69
	3 200.1	-	-	-	-	-
	4 132.1	-	-	-	novel	132
	5 132.2	-	-	-	-	132
10	6 3bf4	3	Yes	3	PTK	ambiguous
	7 7.1	-	-	-	-	7, 11, 97
	8 7.2	2.4	-	-	-	7, 11, 97
	9 cc49	7, 4	Yes	3.6	Kruppel	97, 103
	10 cc43	1.4	Yes	1.8	hypothetical yeast protein	97, 7, 11
15	11 et1807	2.5	Yes	0.7	novel	97, 9
	12 et2106	None	Yes	-	-	95, 39, 38
	13 41.1	detected	Yes	3	homeotic gene	95, 41, 42
	14 67.1	7, 8, 11	Yes	2Kb	cGMP reg.	67
	15 67.2	-	Yes	-	protein	67
20		0				

**20q13 Amplicon Proteins**

As indicated above, this invention also provides for proteins encoded by nucleic acid sequences in the 20q13 amplicon (e.g., SEQ. ID. Nos: 1-10 and 12-13) and subsequences more preferably subsequences of at least 10 amino acids, preferably of at least 20 amino acids, and most preferably of at least 30 amino acids in length. Particularly preferred subsequences are epitopes specific to the 20q13 proteins more preferably epitopes specific to the ZABC1 and 1b1 proteins. Such proteins

include, but are not limited to isolated polypeptides comprising at least 10 contiguous amino acids from a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12-13 or from the polypeptide of SEQ. ID. No. 11 wherein the polypeptide, when presented as an immunogen, elicits the production of an antibody which specifically binds to a polypeptide selected from the group consisting of a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12-13 or from the polypeptide of SEQ. ID. No. 11 and the polypeptide does not bind to antisera raised against a polypeptide selected from the group consisting of a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12 or from the polypeptide of SEQ. ID. No. 11 which has been fully immunosorbed with a polypeptide selected from the group consisting of a polypeptide encoded by the nucleic acids of SEQ. ID No. 1-10 and 12-13 or from the polypeptide of SEQ. ID. No. 11.

A protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ ID NO 11 is determined in an immunoassay. The immunoassay uses a polyclonal antiserum which was raised to the protein of SEQ ID NO 11 (the immunogenic polypeptide). This antiserum is selected to have low cross reactivity against other similar known polypeptides and any such cross reactivity is removed by immunoabsorption prior to use in the immunoassay (e.g., by immunosorption of the antisera with the related polypeptide).

In order to produce antisera for use in an immunoassay, the polypeptide e.g., the polypeptide of SEQ ID NO 11 is isolated as described herein. For example, recombinant protein can be produced in a mammalian or other eukaryotic cell line. An inbred strain of mice is immunized with the protein of SEQ ID NO 11 using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, *supra*). Alternatively, a synthetic polypeptide derived from the sequences disclosed herein and conjugated to a carrier protein is used as an immunogen. Polyclonal sera are collected and titered against the immunogenic polypeptide in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of  $10^4$  or greater are selected and tested for their cross reactivity against known polypeptides using a competitive binding immunoassay such as the one described in Harlow and

Lane, *supra*, at pages 570-573. Preferably more than one known polypeptide is used in this determination in conjunction with the immunogenic polypeptide.

The known polypeptides can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques as described herein.

Immunoassays in the competitive binding format are used for cross reactivity determinations. For example, the immunogenic polypeptide is immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the proteins to compete with the binding of the antisera to the immobilized protein is compared to the immunogenic polypeptide. The percent cross reactivity for the protein is calculated, using standard calculations. Those antisera with less than 10% cross reactivity to known polypeptides are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorption with known polypeptide.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described herein to compare a "target" polypeptide to the immunogenic polypeptide. To make this comparison, the two polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the antisera to the immobilized protein is determined using standard techniques. If the amount of the target polypeptide required is less than twice the amount of the immunogenic polypeptide that is required, then the target polypeptide is said to specifically bind to an antibody generated to the immunogenic protein. As a final determination of specificity, the pooled antisera is fully immunosorbed with the immunogenic polypeptide until no binding to the polypeptide used in the immunosorption is detectable. The fully immunosorbed antisera is then tested for reactivity with the test polypeptide. If no reactivity is observed, then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

Similarly, in a reciprocal experiment, the pooled antisera is immunosorbed with the test polypeptide. If the antisera which remains after the immunosorption does not bind to the immunogenic polypeptide (*i.e.*, the polypeptide

of SEQ ID NO: 11 used to elicit the antisera) then the test polypeptide is specifically bound by the antisera elicited by the immunogenic peptide.

**Detection of 20q13 Abnormalities.**

One of skill in the art will appreciate that the clones and sequence information provided herein can be used to detect amplifications, or other chromosomal abnormalities, at 20q13 in a biological sample. Generally the methods involve hybridization of probes that specifically bind one or more nucleic acid sequences of the target amplicon with nucleic acids present in a biological sample or derived from a biological sample.

As used herein, a biological sample is a sample of biological tissue or fluid containing cells desired to be screened for chromosomal abnormalities (e.g. amplifications or deletions). In a preferred embodiment, the biological sample is a cell or tissue suspected of being cancerous (transformed). Methods of isolating biological samples are well known to those of skill in the art and include, but are not limited to, aspirations, tissue sections, needle biopsies, and the like. Frequently the sample will be a "clinical sample" which is a sample derived from a patient. It will be recognized that the term "sample" also includes supernatant (containing cells) or the cells themselves from cell cultures, cells from tissue culture and other media in which it may be desirable to detect chromosomal abnormalities.

In some embodiments, a chromosome sample is prepared by depositing cells, either as single cell suspensions or as tissue preparation, on solid supports such as glass slides and fixed by choosing a fixative which provides the best spatial resolution of the cells and the optimal hybridization efficiency. In other embodiments, the sample is contacted with an array of probes immobilized on a solid surface.

**Making Probes**

Any of the P1 probes listed in Table 1, the BAC probes listed in Table 2, or the cDNAs disclosed here are suitable for use in detecting the 20q13 amplicon.

Methods of preparing probes are well known to those of skill in the art (see, e.g.

Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989) or *Current Protocols in Molecular Biology*, F. Ausubel *et al.*, ed. Greene Publishing and Wiley-Interscience, New York (1987))

Given the strategy for making the nucleic acids of the present invention, one of skill can construct a variety of vectors and nucleic acid clones containing functionally equivalent nucleic acids. Cloning methodologies to accomplish these ends, and sequencing methods to verify the sequence of nucleic acids are well known in the art. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are found in Berger and Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology* volume 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3,

5 Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, (Sambrook); and *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1994 Supplement) (Ausubel). Product information from manufacturers of biological reagents and experimental equipment also provide information useful in

10 known biological methods. Such manufacturers include the SIGMA chemical company (Saint Louis, MO), R&D systems (Minneapolis, MN), Pharmacia LKB Biotechnology (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersberg, MD), Fluka

15 Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen, San Diego, CA, and Applied Biosystems (Foster City, CA), as well as many other commercial sources known to one of skill.

20

The nucleic acids provided by this invention, whether RNA, cDNA, genomic DNA, or a hybrid of the various combinations, are isolated from biological sources or synthesized *in vitro*. The nucleic acids and vectors of the invention are present in transformed or transfected whole cells, in transformed or transfected cell lysates, or in a partially purified or substantially pure form.

25 *In vitro* amplification techniques suitable for amplifying sequences to provide a nucleic acid, or for subsequent analysis, sequencing or subcloning are known. Examples of techniques sufficient to direct persons of skill through such *in vitro* amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Q $\beta$ -replicase amplification and other RNA polymerase

mediated techniques (e.g., NASBA) are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.*, (1987) U.S. Patent No. 4,683,202; *PCR Protocols A Guide to Methods and Applications* (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) *C&EN* 36-47; *The Journal Of NIH Research* (1991) 3, 81-94; (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86, 1173; Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87, 1874; Lomell *et al.* (1989) *J. Clin. Chem* 35, 1826; Landegren *et al.*, (1988) *Science* 241, 1077-1080; Van Brunt (1990) *Biotechnology* 8, 291-294; Wu and Wallace, (1989) *Gene* 4, 560; Barringer *et al.* (1990) *Gene* 89, 117, and Sooknanan and Malek (1995) *Biotechnology* 13: 563-564. Improved methods of cloning *in vitro* amplified nucleic acids are described in Wallace *et al.*, U.S. Pat. No. 5,426,039. Improved methods of amplifying large nucleic acids are summarized in Cheng *et al.* (1994) *Nature* 369: 684-685 and the references therein.

Nucleic Acids (e.g., oligonucleotides) for *in vitro* amplification methods or for use as gene probes, for example, are typically chemically synthesized according to the solid phase phosphoramidite triester method described by Beaucage and Caruthers (1981), *Tetrahedron Lett.*, 22(20):1859-1862, e.g., using an automated synthesizer, as described in Needham-VanDevanter *et al.* (1984) *Nucleic Acids Res.*, 12:6159-6168. Purification of oligonucleotides, where necessary, is typically performed by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson and Regnier (1983) *J. Chrom.* 255:137-149. The sequence of the synthetic oligonucleotides can be verified using the chemical degradation method of Maxam and Gilbert (1980) in Grossman and Moldave (eds.) Academic Press, New York, *Methods in Enzymology* 65:499-560.

The probes are most easily prepared by combining and labeling one or more of the constructs listed in Tables 1 and 2. Prior to use, the constructs are fragmented to provide smaller nucleic acid fragments that easily penetrate the cell and hybridize to the target nucleic acid. Fragmentation can be by any of a number of methods well known to those of skill in the art. Preferred methods include treatment with a restriction enzyme to selectively cleave the molecules, or alternatively to briefly heat the nucleic acids in the presence of Mg<sup>2+</sup>. Probes are preferably fragmented to an average fragment length ranging from about 50 bp to about 2000 bp,

more preferably from about 100 bp to about 1000 bp and most preferably from about 150 bp to about 500 bp.

Alternatively, probes can be produced by amplifying (e.g. via PCR) selected subsequences from the 20q13 amplicon disclosed herein. The sequences provided herein permit one of skill to select primers that amplify sequences from one or more exons located within the 20q13 amplicon.

Particularly preferred probes include nucleic acids from probes 38, 40, and 79, which corresponds to RMC20C001. In addition, the cDNAs are particularly useful for identifying cells that have increased expression of the corresponding genes, using for instance, Northern blot analysis.

One of skill will appreciate that using the sequence information and clones provided herein, one of skill in the art can isolate the same or similar probes from other human genomic libraries using routine methods (e.g. Southern or Northern Blots).

Similarly, the polypeptides of the invention can be synthetically prepared in a wide variety of well-known ways. For instance, polypeptides of relatively short length can be synthesized in solution or on a solid support in accordance with conventional techniques. See, e.g., Merrifield (1963) *J. Am. Chem. Soc.* 85:2149-2154. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, e.g., Stewart and Young (1984) *Solid Phase Peptide Synthesis*, 2d. ed., Pierce Chemical Co. As described in more detail herein, the polypeptide of the invention are most preferably made using recombinant techniques, e.g., by expressing the polypeptides in host cells and purifying the expressed proteins.

In a preferred embodiment, the polypeptides, or subsequences thereof, are synthesized using recombinant DNA methodology. Generally this involves creating a DNA sequence that encodes the protein, through recombinant, synthetic, or *in vitro* amplification techniques, placing the DNA in an expression cassette under the control of a particular promoter, expressing the protein in a host cell, isolating the expressed protein and, if required, renaturing the protein.

### Labeling Nucleic Acids

Methods of labeling nucleic acids (either probes or sample nucleic acids) are well known to those of skill in the art. Preferred labeled labels are those that are suitable for use in *in situ* hybridization. The nucleic acid probes or samples of the invention may be detectably labeled prior to the hybridization reaction.

Alternatively, a detectable label which binds to the hybridization product may be used. Such detectable labels include any material having a detectable physical or chemical property and have been well-developed in the field of immunoassays.

As used herein, a "label" is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Useful labels in the present invention include radioactive labels (e.g.  $^{32}\text{P}$ ,  $^{125}\text{I}$ ,  $^{14}\text{C}$ ,  $^3\text{H}$ , and  $^{35}\text{S}$ ), fluorescent dyes (e.g. fluorescein, rhodamine, Texas Red, etc.), electron-dense reagents (e.g. gold), enzymes (as commonly used in an ELISA), colorimetric labels (e.g. colloidal gold), magnetic labels (e.g. Dynabeads<sup>TM</sup>), and the like. Examples of labels which are not directly detected but are detected through the use of directly detectable label include biotin and dioxigenin as well as haptens and proteins for which labeled antisera or monoclonal antibodies are available.

The particular label used is not critical to the present invention, so long as it does not interfere with the *in situ* hybridization of the stain. However, stains directly labeled with fluorescent labels (e.g. fluorescein-12-dUTP, Texas Red-5-dUTP, etc.) are preferred for chromosome hybridization.

A direct labeled probe, as used herein, is a probe to which a detectable label is attached. Because the direct label is already attached to the probe, no subsequent steps are required to associate the probe with the detectable label. In contrast, an indirect labeled probe is one which bears a moiety to which a detectable label is subsequently bound, typically after the probe is hybridized with the target nucleic acid.

In addition the label must be detectible in as low copy number as possible thereby maximizing the sensitivity of the assay and yet be detectible above any background signal. Finally, a label must be chosen that provides a highly localized signal thereby providing a high degree of spatial resolution when physically

mapping the stain against the chromosome. Particularly preferred fluorescent labels include fluorescein-12-dUTP and Texas Red-5-dUTP.

The labels may be coupled to the probes in a variety of means known to those of skill in the art. In a preferred embodiment the nucleic acid probes will be labeled using nick translation or random primer extension (Rigby, *et al.* *J. Mol. Biol.*, 113: 237 (1977) or Sambrook, *et al.*, *Molecular Cloning - A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1985)).

One of skill in the art will appreciate that the probes of this invention need not be absolutely specific for the targeted 20q13 region of the genome. Rather, the probes are intended to produce "staining contrast". "Contrast" is quantified by the ratio of the probe intensity of the target region of the genome to that of the other portions of the genome. For example, a DNA library produced by cloning a particular chromosome (*e.g.* chromosome 7) can be used as a stain capable of staining the entire chromosome. The library contains both sequences found only on that chromosome, and sequences shared with other chromosomes. Roughly half the chromosomal DNA falls into each class. If hybridization of the whole library were capable of saturating all of the binding sites on the target chromosome, the target chromosome would be twice as bright (contrast ratio of 2) as the other chromosomes since it would contain signal from the both the specific and the shared sequences in the stain, whereas the other chromosomes would only be stained by the shared sequences. Thus, only a modest decrease in hybridization of the shared sequences in the stain would substantially enhance the contrast. Thus contaminating sequences which only hybridize to non-targeted sequences, for example, impurities in a library, can be tolerated in the stain to the extent that the sequences do not reduce the staining contrast below useful levels.

#### **Detecting the 20q13 Amplicon.**

As explained above, detection of amplification in the 20q13 amplicon is indicative of the presence and/or prognosis of a large number of cancers. These include, but are not limited to breast, ovary, bladder, head and neck, and colon.

In a preferred embodiment, a 20q13 amplification is detected through the hybridization of a probe of this invention to a target nucleic acid (*e.g.* a chromosomal sample) in which it is desired to screen for the amplification. Suitable

hybridization formats are well known to those of skill in the art and include, but are not limited to, variations of Southern Blots, *in situ* hybridization and quantitative amplification methods such as quantitative PCR (see, e.g. Sambrook, *supra.*, Kallioniemi *et al.*, *Proc. Natl Acad Sci USA*, 89: 5321-5325 (1992), and *PCR Protocols, A Guide to Methods and Applications*, Innis *et al.*, Academic Press, Inc. N.Y., (1990)).

**In situ Hybridization.**

In a preferred embodiment, the 20q13 amplicon is identified using *in situ* hybridization. Generally, *in situ* hybridization comprises the following major steps: (1) fixation of tissue or biological structure to analyzed; (2) prehybridization treatment of the biological structure to increase accessibility of target DNA, and to reduce nonspecific binding; (3) hybridization of the mixture of nucleic acids to the nucleic acid in the biological structure or tissue; (4) posthybridization washes to remove nucleic acid fragments not bound in the hybridization and (5) detection of the hybridized nucleic acid fragments. The reagent used in each of these steps and their conditions for use vary depending on the particular application.

In some applications it is necessary to block the hybridization capacity of repetitive sequences. In this case, human genomic DNA is used as an agent to block such hybridization. The preferred size range is from about 200 bp to about 20 1000 bases, more preferably between about 400 to about 800 bp for double stranded. nick translated nucleic acids.

Hybridization protocols for the particular applications disclosed here are described in Pinkel *et al.* *Proc. Natl. Acad. Sci. USA*, 85: 9138-9142 (1988) and in EPO Pub. No. 430,402. Suitable hybridization protocols can also be found in 25 *Methods of Molecular Biology Vol. 33: In Situ Hybridization Protocols*, K.H.A. Choo, ed., Humana Press, Totowa, New Jersey, (1994). In a particularly preferred embodiment, the hybridization protocol of Kallioniemi *et al.*, *Proc. Natl Acad Sci USA*, 89: 5321-5325 (1992) is used.

Typically, it is desirable to use dual color FISH, in which two probes 30 are utilized, each labeled by a different fluorescent dye. A test probe that hybridizes to the region of interest is labeled with one dye, and a control probe that hybridizes to a different region is labeled with a second dye. A nucleic acid that hybridizes to a

stable portion of the chromosome of interest, such as the centromere region, is often most useful as the control probe. In this way, differences between efficiency of hybridization from sample to sample can be accounted for.

The FISH methods for detecting chromosomal abnormalities can be performed on nanogram quantities of the subject nucleic acids. Paraffin embedded tumor sections can be used, as can fresh or frozen material. Because FISH can be applied to the limited material, touch preparations prepared from uncultured primary tumors can also be used (see, e.g., Kallioniemi, A. et al., *Cytogenet. Cell Genet.* 60: 190-193 (1992)). For instance, small biopsy tissue samples from tumors can be used for touch preparations (see, e.g., Kallioniemi, A. et al., *Cytogenet. Cell Genet.* 60: 190-193 (1992)). Small numbers of cells obtained from aspiration biopsy or cells in bodily fluids (e.g., blood, urine, sputum and the like) can also be analyzed. For prenatal diagnosis, appropriate samples will include amniotic fluid and the like.

#### Other Formats

A number of hybridization formats are useful in the invention. For instance, Southern hybridizations can be used. In a Southern Blot, a genomic or cDNA (typically fragmented and separated on an electrophoretic gel) is hybridized to a probe specific for the target region. Comparison of the intensity of the hybridization signal from the probe for the target region (e.g., 20q13) with the signal from a probe directed to a control (non amplified) such as centromeric DNA, provides an estimate of the relative copy number of the target nucleic acid.

Other formats use arrays of probes or targets to which nucleic acid samples are hybridized as described, for instance, in WO 96/17958. As used herein, a "nucleic acid array" is a plurality of target elements, each comprising one or more target nucleic acid molecules immobilized on a solid surface to which probe nucleic acids are hybridized. Target nucleic acids of a target element typically have their origin in the 20q13 amplicon. The target nucleic acids of a target element may, for example, contain sequence from specific genes or clones disclosed here. Target elements of various dimensions can be used in the arrays of the invention. Generally, smaller, target elements are preferred. Typically, a target element will be less than about 1cm in diameter. Generally element sizes are from 1 $\mu$ m to about 3mm, preferably between about 5  $\mu$ m and about 1mm.

The target elements of the arrays may be arranged on the solid surface at different densities. The target element densities will depend upon a number of factors, such as the nature of the label, the solid support, and the like. One of skill will recognize that each target element may comprise a mixture of target nucleic acids of different lengths and sequences. Thus, for example, a target element may contain more than one copy of a cloned piece of DNA, and each copy may be broken into fragments of different lengths. The length and complexity of the target sequences of the invention is not critical to the invention. One of skill can adjust these factors to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among different genes or genomic locations. Typically, the target sequences will have a complexity between about 1 kb and about 1 Mb, sometimes 10kb and about 500kb, and usually from about 50kb to about 150kb.

**Detecting Mutations in Genes from the 20q13 Amplicon**

The cDNA sequences disclosed here can also be used for detecting mutations (*e.g.*, substitutions, insertions, and deletions) within the corresponding endogenous genes. One of skill will recognize that the nucleic acid hybridization techniques generally described above can be adapted to detect such much mutations.

For instance, oligonucleotide probes that distinguish between mutant and wild-type forms of the target gene can be used in standard hybridization assays. In some embodiments, amplification (*e.g.*, using PCR) can be used to increase copy number of the target sequence prior to hybridization.

**Assays for detecting 20q13 amplicon proteins.**

As indicated above, this invention identifies protein products of genes in the 20q13 amplicon that are associated with various cancers. In particular, it was shown that 20q13 proteins were overexpressed in various cancers. The presence or absence and/or level of expression of 20q13 proteins can be indicative of the presence, absence, or extent of a cancer. Thus, 20q13 proteins can provide useful diagnostic markers.

The 20q13 amplicon proteins (*e.g.*, ZABC1 or 1b1) can be detected and quantified by any of a number of means well known to those of skill in the art.

These may include analytic biochemical methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, or various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunolectrophoresis, radioimmunoassay(RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, western blotting, and the like.

In one preferred embodiment, the 20q13 amplicon proteins are detected in an electrophoretic protein separation such as a one dimensional or two-dimensional electrophoresis, while in a most preferred embodiment, the 20q13 amplicon proteins are detected using an immunoassay.

As used herein, an immunoassay is an assay that utilizes an antibody to specifically bind to the analyte (e.g., ZABC1 or 1b1 proteins). The immunoassay is thus characterized by detection of specific binding of a 20q13 amplicon protein to an anti-20q13 amplicon antibody (e.g., anti-ZABC1 or anti-1b1) as opposed to the use of other physical or chemical properties to isolate, target, and quantify the analyte.

The collection of biological sample and subsequent testing for 20q13 amplicon protein(s) is discussed in more detail below.

#### A) Sample Collection and Processing

The 20q13 amplicon proteins are preferably quantified in a biological sample derived from a mammal, more preferably from a human patient or from a porcine, murine, feline, canine, or bovine. As used herein, a biological sample is a sample of biological tissue or fluid that contains a 20q13 amplicon protein concentration that may be correlated with a 20q13 amplification. Particularly preferred biological samples include, but are not limited to biological fluids such as blood or urine, or tissue samples including, but not limited to tissue biopsy (e.g., needle biopsy) samples.

The biological sample may be pretreated as necessary by dilution in an appropriate buffer solution or concentrated, if desired. Any of a number of standard

aqueous buffer solutions, employing one of a variety of buffers, such as phosphate, Tris, or the like, at physiological pH can be used.

**B) Electrophoretic Assays.**

As indicated above, the presence or absence of 20q13 amplicon proteins in a biological tissue may be determined using electrophoretic methods. Means of detecting proteins using electrophoretic techniques are well known to those of skill in the art (see generally, R. Scopes (1982) *Protein Purification*, Springer-Verlag, N.Y.; Deutscher, (1990) *Methods in Enzymology Vol. 182: Guide to Protein Purification*, Academic Press, Inc., N.Y.). In a preferred embodiment, the 20q13 amplicon proteins are detected using one-dimensional or two-dimensional electrophoresis. A particularly preferred two-dimensional electrophoresis separation relies on isoelectric focusing (IEF) in immobilized pH gradients for one dimension and polyacrylamide gels for the second dimension. Such assays are described in the cited references and by Patton *et al.* (1990) *Biotechniques* 8: 518.

**C) Immunological Binding Assays.**

In a preferred embodiment, the 20q13 amplicon are detected and/or quantified using any of a number of well recognized immunological binding assays (see, e.g., U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general immunoassays, see also *Methods in Cell Biology Volume 37: Antibodies in Cell Biology*, Asai, ed. Academic Press, Inc. New York (1993); *Basic and Clinical Immunology* 7th Edition, Stites & Terr, eds. (1991).

Immunological binding assays (or immunoassays) typically utilize a "capture agent" to specifically bind to and often immobilize the analyte (in this case 20q13 amplicon). The capture agent is a moiety that specifically binds to the analyte. In a preferred embodiment, the capture agent is an antibody that specifically binds 20q13 amplicon protein(s).

The antibody (e.g., anti-ZABC1 or anti-1b1) may be produced by any of a number of means well known to those of skill in the art (see, e.g. *Methods in Cell Biology Volume 37: Antibodies in Cell Biology*, Asai, ed. Academic Press, Inc. New York (1993); and *Basic and Clinical Immunology* 7th Edition, Stites & Terr, eds. (1991)). The antibody may be a whole antibody or an antibody fragment. It may be polyclonal or monoclonal, and it may be produced by challenging an organism

(e.g. mouse, rat, rabbit, etc.) with a 20q13 amplicon protein or an epitope derived therefrom. Alternatively, the antibody may be produced *de novo* using recombinant DNA methodology. The antibody can also be selected from a phage display library screened against 20q13 amplicon (see, e.g. Vaughan *et al.* (1996) *Nature Biotechnology*, 14: 309-314 and references therein).

5 Immunoassays also often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Thus, the labeling agent may be a labeled 20q13 amplicon protein or a  
10 labeled anti-20q13 amplicon antibody. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/20q13 amplicon protein complex. In a preferred embodiment, the labeling agent is a second human 20q13 amplicon protein antibody bearing a label. Alternatively, the second 20q13 amplicon protein antibody may lack a label, but it may, in turn, be  
15 bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

Other proteins capable of specifically binding immunoglobulin constant  
20 regions, such as protein A or protein G may also be used as the label agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species. See, generally Kronval, *et al.*, *J. Immunol.*, 111:1401-1406 (1973), and Akerstrom, *et al.*, *J. Immunol.*, 135:2589-2542 (1985).

25 Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at  
30 ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

### 11 Non-Competitive Assay Formats

Immunoassays for detecting 20q13 amplicon proteins may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (in this case 20q13 amplicon) is directly measured. In one preferred "sandwich" assay, for example, the capture agent (anti-20q13 amplicon protein antibodies) can be bound directly to a solid substrate where they are immobilized. These immobilized antibodies then capture 20q13 amplicon protein present in the test sample. The 20q13 amplicon protein thus immobilized is then bound by a labeling agent, such as a second human 20q13 amplicon protein antibody bearing a label. Alternatively, the second 20q13 amplicon protein antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

## 2. Competitive assay formats.

In competitive assays, the amount of analyte (20q13 amplicon protein) present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte (20q13 amplicon proteins such as ZABC1 or 1b1 protein) displaced (or competed away) from a capture agent (e.g., anti-ZABC1 or anti-1b1 antibody) by the analyte present in the sample. In one competitive assay, a known amount of, in this case, 20q13 amplicon protein is added to the sample and the sample is then contacted with a capture agent, in this case an antibody that specifically binds 20q13 amplicon protein. The amount of 20q13 amplicon protein bound to the antibody is inversely proportional to the concentration of 20q13 amplicon protein present in the sample.

In a particularly preferred embodiment, the anti-20q13 protein antibody is immobilized on a solid substrate. The amount of 20q13 amplicon protein bound to the antibody may be determined either by measuring the amount of 20q13 amplicon present in an 20q13 amplicon protein/antibody complex, or alternatively by measuring the amount of remaining uncomplexed 20q13 amplicon protein. The amount of 20q13 amplicon protein may be detected by providing a labeled 20q13 amplicon protein.

A hapten inhibition assay is another preferred competitive assay. In this assay a known analyte, in this case 20q13 amplicon protein is immobilized on a

solid substrate. A known amount of anti-20q13 amplicon protein antibody is added to the sample, and the sample is then contacted with the immobilized 20q13 amplicon protein. In this case, the amount of anti-20q13 amplicon protein antibody bound to the immobilized 20q13 amplicon protein is inversely proportional to the amount of 20q13 amplicon protein present in the sample. Again the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

### 10      3. Other Assay Formats

In a particularly preferred embodiment, Western blot (immunoblot) analysis is used to detect and quantify the presence of 20q13 amplicon protein in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind 20q13 amplicon protein. The anti-20q13 amplicon protein antibodies specifically bind to 20q13 amplicon protein on the solid support. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the anti-20q13 amplicon protein.

Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (e.g., antibodies) and release encapsulated reagents or markers. The released chemicals are then detected according to standard techniques (see, Monroe *et al.* (1986) *Amer. Clin. Prod. Rev.* 5:34-41).

#### 25      D) Reduction of Non-Specific Binding.

One of skill in the art will appreciate that it is often desirable to reduce non-specific binding in immunoassays. Particularly, where the assay involves an antigen or antibody immobilized on a solid substrate it is desirable to minimize the amount of non-specific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art. Typically, this involves coating the substrate with a proteinaceous composition. In particular, protein compositions

such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used with powdered milk being most preferred.

E) Labels.

The particular label or detectable group used in the assay is not a critical aspect of the invention, so long as it does not significantly interfere with the specific binding of the antibody used in the assay. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, most any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads (e.g. Dynabeads<sup>TM</sup>), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, and the like), radiolabels (e.g., <sup>3</sup>H, <sup>125</sup>I, <sup>35</sup>S, <sup>14</sup>C, or <sup>32</sup>P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (e.g., streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, *etc.* Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal producing systems which may be used, see, U.S. Patent No. 4,391,904).

Means of detecting labels are well known to those of skill in the art.

Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

### G) Substrates.

As mentioned above, depending upon the assay, various components, including the antigen, target antibody, or anti-human antibody, may be bound to a solid surface. Many methods for immobilizing biomolecules to a variety of solid surfaces are known in the art. For instance, the solid surface may be a membrane (*e.g.*, nitrocellulose), a microtiter dish (*e.g.*, PVC, polypropylene, or polystyrene), a test tube (glass or plastic), a dipstick (*e.g.* glass, PVC, polypropylene, polystyrene,

latex, and the like), a microcentrifuge tube, or a glass or plastic bead. The desired component may be covalently bound or noncovalently attached through nonspecific bonding.

A wide variety of organic and inorganic polymers, both natural and synthetic may be employed as the material for the solid surface. Illustrative polymers include polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and the like. Other materials which may be employed, include paper, glasses, ceramics, metals, metalloids, semiconductive materials, cements or the like. In addition, are included substances that form gels, such as proteins (e.g., gelatins), lipopolysaccharides, silicates, agarose and polyacrylamides can be used. Polymers which form several aqueous phases, such as dextrans, polyalkylene glycols or surfactants, such as phospholipids, long chain (12-24 carbon atoms) alkyl ammonium salts and the like are also suitable. Where the solid surface is porous, various pore sizes may be employed depending upon the nature of the system.

In preparing the surface, a plurality of different materials may be employed, particularly as laminates, to obtain various properties. For example, protein coatings, such as gelatin can be used to avoid non-specific binding, simplify covalent conjugation, enhance signal detection or the like.

If covalent bonding between a compound and the surface is desired, the surface will usually be polyfunctional or be capable of being polyfunctionalized. Functional groups which may be present on the surface and used for linking can include carboxylic acids, aldehydes, amino groups, cyano groups, ethylenic groups, hydroxyl groups, mercapto groups and the like. The manner of linking a wide variety of compounds to various surfaces is well known and is amply illustrated in the literature. See, for example, *Immobilized Enzymes*, Ichiro Chibata, Halsted Press, New York, 1978, and Cuatrecasas (1970) *J. Biol. Chem.* 245 3059).

In addition to covalent bonding, various methods for noncovalently binding an assay component can be used. Noncovalent binding is typically nonspecific absorption of a compound to the surface. Typically, the surface is blocked with a second compound to prevent nonspecific binding of labeled assay

components. Alternatively, the surface is designed such that it nonspecifically binds one component but does not significantly bind another. For example, a surface bearing a lectin such as Concanavalin A will bind a carbohydrate containing compound but not a labeled protein that lacks glycosylation. Various solid surfaces for use in noncovalent attachment of assay components are reviewed in U.S. Patent Nos. 4,447,576 and 4,254,082.

#### Kits Containing 20q13 Amplicon Probes.

This invention also provides diagnostic kits for the detection of chromosomal abnormalities at 20q13. In a preferred embodiment, the kits include one or more probes to the 20q13 amplicon and/or antibodies to a 20q13 amplicon (e.g., anti-ZABC1 or anti-1b1) described herein. The kits can additionally include blocking probes, instructional materials describing how to use the kit contents in detecting 20q13 amplicons. The kits may also include one or more of the following: various labels or labeling agents to facilitate the detection of the probes, reagents for the hybridization including buffers, a metaphase spread, bovine serum albumin (BSA) and other blocking agents, sampling devices including fine needles, swabs, aspirators and the like, positive and negative hybridization controls and so forth.

#### Expression of cDNA clones

One may express the desired polypeptides encoded by the cDNA clones disclosed here, or by subcloning cDNA portions of genomic sequences in a recombinantly engineered cell such as bacteria, yeast, insect (especially employing baculoviral vectors), or mammalian cell. It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of the cDNAs. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of natural or synthetic nucleic acids encoding polypeptides of the invention will typically be achieved by operably linking the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding the polypeptides. To

obtain high level expression of a cloned gene, it is desirable to construct expression plasmids which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator.

5 Examples of regulatory regions suitable for this purpose in *E. coli* are the promoter and operator region of the *E. coli* tryptophan biosynthetic pathway as described by Yanofsky, C., 1984, J. Bacteriol., 158:1018-1024 and the leftward promoter of phage lambda ( $P_L$ ) as described by Herskowitz, I. and Hagen, D., 1980, Ann. Rev. Genet., 14:399-445. The inclusion of selection markers in DNA vectors 10 transformed in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol. Expression systems are available using *E. coli*, *Bacillus* sp. (Palva, I et al., 1983, Gene 22:229-235; Mosbach, K. et al. Nature, 302:543-545 and *Salmonella*. *E. coli* systems are preferred.

15 The polypeptides produced by prokaryote cells may not necessarily fold properly. During purification from *E. coli*, the expressed polypeptides may first be denatured and then renatured. This can be accomplished by solubilizing the bacterially produced proteins in a chaotropic agent such as guanidine HCl and reducing all the cysteine residues with a reducing agent such as beta-mercaptoethanol. 20 The polypeptides are then renatured, either by slow dialysis or by gel filtration. U.S. Patent No. 4,511,503.

A variety of eukaryotic expression systems such as yeast, insect cell lines and mammalian cells, are known to those of skill in the art. As explained briefly below, the polypeptides may also be expressed in these eukaryotic systems.

25 Synthesis of heterologous proteins in yeast is well known and described. *Methods in Yeast Genetics*, Sherman, F., et al., Cold Spring Harbor Laboratory, (1982) is a well recognized work describing the various methods available to produce the polypeptides in yeast. A number of yeast expression plasmids like YEp6, YEp13, YEp4 can be used as vectors. A gene of interest can be fused to any 30 of the promoters in various yeast vectors. The above-mentioned plasmids have been fully described in the literature (Botstein, et al., 1979, Gene, 8:17-24; Broach, et al., 1979, Gene, 8:121-133).

Illustrative of cell cultures useful for the production of the polypeptides are cells of insect or mammalian origin. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. Illustrative examples of mammalian cell lines include VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, W138, BHK, Cos-7 or MDCK cell lines.

As indicated above, the vector, e. g., a plasmid, which is used to transform the host cell, preferably contains DNA sequences to initiate transcription and sequences to control the translation of the antigen gene sequence. These sequences are referred to as expression control sequences. When the host cell is of insect or mammalian origin illustrative expression control sequences are often obtained from the SV-40 promoter (Science, 222:524-527, 1983), the CMV I.E. Promoter (Proc. Natl. Acad. Sci. 81:659-663, 1984) or the metallothionein promoter (Nature 296:39-42, 1982). The cloning vector containing the expression control sequences is cleaved using restriction enzymes and adjusted in size as necessary or desirable and ligated with the desired DNA by means well known in the art.

As with yeast, when higher animal host cells are employed, polyadenylation or transcription terminator sequences from known mammalian genes need to be incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VPI intron from SV40 (Sprague, J. et al., 1983, J. Virol. 45: 773-781).

Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors. Saveria-Campo, M., 1985, "Bovine Papilloma virus DNA a Eukaryotic Cloning Vector" in DNA Cloning Vol. II a Practical Approach Ed. D.M. Glover, IRL Press, Arlington, Virginia pp. 213-238.

#### Therapeutic and other uses of cDNAs and their gene products

The cDNA sequences and the polypeptide products of the invention can be used to modulate the activity of the gene products of the endogenous genes corresponding to the cDNAs. By modulating activity of the gene products, pathological conditions associated with their expression or lack of expression can be

treated. Any of a number of techniques well known to those of skill in the art can be used for this purpose.

The cDNAs of the invention are particularly used for the treatment of various cancers such as cancers of the breast, ovary, bladder, head and neck, and colon. Other diseases may also be treated with the sequences of the invention. For instance, as noted above, GCAP (SEQ. ID. No. 6) encodes a guanino cyclase activating protein which is involved in the biosynthesis of cyclic AMP. Mutations in genes involved in the biosynthesis of cyclic AMP are known to be associated with hereditary retinal degenerative diseases. These diseases are a group of inherited conditions in which progressive, bilateral degeneration of retinal structures leads to loss of retinal function. These diseases include age-related macular degeneration, a leading cause of visual impairment in the elderly; Leber's congenital amaurosis, which causes its victims to be born blind; and retinitis pigmentosa ("RP"), one of the most common forms of inherited blindness. RP is the name given to those inherited retinopathies which are characterized by loss of retinal photoreceptors (rods and cones), with retinal electrical responses to light flashes (i.e. electroretinograms, or "ERGs") that are reduced in amplitude.

The mechanism of retinal photoreceptor loss or cell death in different retinal degenerations is not fully understood. Mutations in a number of different genes have been identified as the primary genetic lesion in different forms of human RP. Affected genes include rhodopsin, the alpha and beta subunits of cGMP photodiesterase, and peripherin-RDS (Dryja, T. P. et al., *Invest. Ophthalmol. Vis. Sci.* 36, 1197-1200 (1995)). In all cases the manifestations of the disorder regardless of the specific primary genetic mutation is similar, resulting in photoreceptor cell degeneration and blindness.

Studies on animal models of retinal degeneration have been the focus of many laboratories during the last decade. The mechanisms that are altered in some of the mutations leading to blindness have been elucidated. This would include the inherited disorders of the *rd* mouse. The *rd* gene encodes the beta subunit of cGMP-phosphodiesterase (PDE) (Bowes, C. et al., *Nature* 347, 677-680 (1990)), an enzyme of fundamental importance in normal visual function because it is a key component in the cascade of events that takes place in phototransduction.

The polypeptides encoded by the cDNAs of the invention can be used as immunogens to raise antibodies either polyclonal or monoclonal. The antibodies can be used to detect the polypeptides for diagnostic purposes, as therapeutic agents to inhibit the polypeptides, or as targeting moieties in immunotoxins. The production of monoclonal antibodies against a desired antigen is well known to those of skill in the art and is not reviewed in detail here.

Those skilled in the art recognize that there are many methods for production and manipulation of various immunoglobulin molecules. As used herein, the terms "immunoglobulin" and "antibody" refer to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. Immunoglobulins may exist in a variety of forms besides antibodies, including for example, Fv, Fab, and F(ab)<sub>2</sub>, as well as in single chains. To raise monoclonal antibodies, antibody-producing cells obtained from immunized animals (e.g., mice) are immortalized and screened, or screened first for the production of the desired antibody and then immortalized. For a discussion of general procedures of monoclonal antibody production see Harlow and Lane, *Antibodies, A Laboratory Manual* Cold Spring Harbor Publications, N.Y. (1988).

The antibodies raised by these techniques can be used in immunodiagnostic assays to detect or quantify the expression of gene products from the nucleic acids disclosed here. For instance, labeled monoclonal antibodies to polypeptides of the invention can be used to detect expression levels in a biological sample. For a review of the general procedures in diagnostic immunoassays, see *Basic and Clinical Immunology* 7th Edition D. Stites and A. Terr ed. (1991).

The polynucleotides of the invention are particularly useful for gene therapy techniques well known to those skilled in the art. Gene therapy as used herein refers to the multitude of techniques by which gene expression may be altered in cells. Such methods include, for instance, introduction of DNA encoding ribozymes or antisense nucleic acids to inhibit expression as well as introduction of functional wild-type genes to replace mutant genes (e.g., using wild-type GCAP genes to treat retinal degeneration). A number of suitable viral vectors are known. Such vectors include retroviral vectors (see Miller, *Curr. Top. Microbiol. Immunol.* 158: 1-24 (1992); Salmons and Gunzburg, *Human Gene Therapy* 4: 129-141 (1993); Miller

et al., *Methods in Enzymology* 217: 581-599, (1994)) and adeno-associated vectors (reviewed in Carter, *Curr. Opinion Biotech.* 3: 533-539 (1992); Muzyczka, *Curr. Top. Microbiol. Immunol.* 158: 97-129 (1992)). Other viral vectors that may be used within the methods include adenoviral vectors, herpes viral vectors and Sindbis viral vectors, as generally described in, e.g., Jolly, *Cancer Gene Therapy* 1:51-64 (1994); Latchman, *Molec. Biotechnol.* 2:179-195 (1994); and Johanning et al., *Nucl. Acids Res.* 23:1495-1501 (1995).

Delivery of nucleic acids linked to a heterologous promoter-enhancer element via liposomes is also known (see, e.g., Brigham, et al. (1989) *Am. J. Med. Sci.*, 298:278-281; Nabel, et al. (1990) *Science*, 249:1285-1288; Hazinski, et al. (1991) *Am. J. Resp. Cell Molec. Biol.*, 4:206-209; and Wang and Huang (1987) *Proc. Natl. Acad. Sci. (USA)*, 84:7851-7855); coupled to ligand-specific, cation-based transport systems (Wu and Wu (1988) *J. Biol. Chem.*, 263:14621-14624). Naked DNA expression vectors have also been described (Nabel et al. (1990), *supra*); Wolff et al. (1990) *Science*, 247:1465-1468).

The nucleic acids and encoded polypeptides of the invention can be used directly to inhibit the endogenous genes or their gene products. For instance, Inhibitory nucleic acids may be used to specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA duplex is formed. These nucleic acids are often termed "antisense" because they are usually complementary to the sense or coding strand of the gene, although approaches for use of "sense" nucleic acids have also been developed. The term "inhibitory nucleic acids" as used herein, refers to both "sense" and "antisense" nucleic acids. Inhibitory nucleic acid methods encompass a number of different approaches to altering expression of specific genes that operate by different mechanisms.

In brief, inhibitory nucleic acid therapy approaches can be classified into those that target DNA sequences, those that target RNA sequences (including pre-mRNA and mRNA), those that target proteins (sense strand approaches), and those that cause cleavage or chemical modification of the target nucleic acids (ribozymes). These different types of inhibitory nucleic acid technology are described, for instance, in Helene, C. and Toulme, J. (1990) *Biochim. Biophys. Acta.*, 1049:99-125.

Inhibitory nucleic acid complementary to regions of c-myc mRNA has been shown to inhibit c-myc protein expression in a human promyelocytic leukemia cell line, HL60, which overexpresses the c-myc protooncogene. See Wickstrom E.L., et al., (1988) *PNAS (USA)*, 85:1028-1032 and Harel-Bellan, A., et al., (1988) *Exp. Med.*, 168:2309-2318.

The encoded polypeptides of the invention can also be used to design molecules (peptidic or nonpeptidic) that inhibit the endogenous proteins by, for instance, inhibiting interaction between the protein and a second molecule specifically recognized by the protein. Methods for designing such molecules are well known to those skilled in the art.

For instance, polypeptides can be designed which have sequence identity with the encoded proteins or may comprise modifications (conservative or non-conservative) of the sequences. The modifications can be selected, for example, to alter their *in vivo* stability. For instance, inclusion of one or more D-amino acids in the peptide typically increases stability, particularly if the D-amino acid residues are substituted at one or both termini of the peptide sequence.

The polypeptides can also be modified by linkage to other molecules. For example, different N- or C-terminal groups may be introduced to alter the molecule's physical and/or chemical properties. Such alterations may be utilized to affect, for example, adhesion, stability, bio-availability, localization or detection of the molecules. For diagnostic purposes, a wide variety of labels may be linked to the terminus, which may provide, directly or indirectly, a detectable signal. Thus, the polypeptides may be modified in a variety of ways for a variety of end purposes while still retaining biological activity.

**EXAMPLES**

The following examples are offered to illustrate, but not to limit the present invention.

**Example 1****5 PROGNOSTIC IMPLICATIONS OF AMPLIFICATION OF CHROMOSOMAL REGION 20q13 IN BREAST CANCER****Patients and tumor material.**

Tumor samples were obtained from 152 women who underwent surgery for breast cancer between 1987 and 1992 at the Tampere University or City Hospitals.

10 One hundred and forty-two samples were from primary breast carcinomas and 11 from metastatic tumors. Specimens from both the primary tumor and a local metastasis were available from one patient. Ten of the primary tumors that were either *in situ* or mucinous carcinomas were excluded from the material, since the specimens were considered inadequate for FISH studies. Of the remaining 132  
15 primary tumors, 128 were invasion ductal and 4 lobular carcinomas. The age of the patients ranged from 29 to 92 years (mean 61). Clinical follow-up was available from 129 patients. Median follow-up period was 45 months (range 1.4-1.77 months). Radiation therapy was given to 77 of the 129 patients (51 patients with positive and 26 with negative lymph nodes), and systemic adjuvant therapy to 36 patients (33 with  
20 endocrine and 3 with cytotoxic chemotherapy). Primary tumor size and axillary node involvement were determined according to the tumor-node metastasis (TNM) classification. The histopathological diagnosis was evaluated according to the World Health Organization (11). The carcinomas were graded on the basis of the tubular arrangement of cancer cells, nuclear atypia, and frequency of mitotic or  
25 hyperchromatic nuclear figures according to Bloom and Richardson, *Br. J. Cancer*, 11: 359-377 (1957).

Surgical biopsy specimens were frozen at -70°C within 15 minutes of removal. Cryostat sections (5-6 µm) were prepared for intraoperative histopathological diagnosis, and additional thin sections were cut for immunohistochemical studies. One adjacent 200 µm thick section was cut for DNA flow cytometric and FISH studies.

Cell preparation for FISH.

After histological verification that the biopsy specimens contained a high proportion of tumor cells, nuclei were isolated from 200  $\mu\text{m}$  frozen sections according to a modified Vindelov procedure for DNA flow cytometry, fixed and dropped on slides for FISH analysis as described by Hytyinen *et al.*, *Cytometry* 16: 93-99 (1994). Foreskin fibroblasts were used as negative controls in amplification studies and were prepared by harvesting cells at confluence to obtain G1 phase enriched interphase nuclei. All samples were fixed in methanol-acetic-acid (3:1).

Probes.

Five probes mapping to the 20q13 region were used (see Stokke, *et al.*, *Genomics*, 26: 134-137 (1995)). The probes included PI-clones for melanocortin-3-receptor (probe MC3R, fractional length from p-arm telomere (Flpter 0.81) and phosphoenolpyruvate carboxy kinase (PCK, Flpter 0.84), as well as anonymous cosmid clones RMC20C026 (Flpter 0.79). In addition, RMC20C001 (Flpter 0.825) and RMC20C030 (Flpter 0.85) were used. Probe RMC20C001 was previously shown to define the region of maximum amplification (Tanner *et al.*, *Cancer Res*, 54: 4257-4260 (1994)). One cosmid probe mapping to the proximal p-arm, RMC20C038 (FLpter 0.237) was used as a chromosome-specific reference probe. Test probes were labeled with biotin-14-dATP and the reference probe with digoxigenin-11-dUTP using nick translation (Kallioniemi *et al.*, *Proc. Natl Acad Sci USA*, 89: 5321-5325 (1992)).

Fluorescence in situ hybridization.

Two-color FISH was performed using biotin-labeled 20q13-specific probes and digoxigenin-labelled 20p reference probe essentially as described (*Id.*). Tumor samples were postfixed in 4% paraformaldehyde/phosphate-buffered saline for 5 min at 4°C prior to hybridization, dehydrated in 70%, 85% and 100% ethanol, air dried, and incubated for 30 min at 80°C. Slides were denatured in a 70% formamide/2x standard saline citrate solution at 72-74°C for 3 min, followed by a proteinase K digestion (0.5  $\mu\text{g}/\text{ml}$ ). The hybridization mixture contained 18 ng of each of the labeled probes and 10  $\mu\text{g}$  human placental DNA. After hybridization, the probes were detected immunochemically with avidin-FITC and anti-digoxigenin

Rhodamine. Slides were counterstained with 0.2  $\mu$ M 4,6-diamidino-2-phenylindole (DAPI) in an antifade solution.

**Fluorescence microscopy and scoring of signals in interphase nuclei.**

A Nikon fluorescence microscope equipped with double band-pass filters (Chromatechnology, Brattleboro, Vermont, USA) and 63 x objective (NA 1.3) was used for simultaneous visualization of FITC and Rhodamine signals. At least 50 non-overlapping nuclei with intact morphology based on the DAPI counterstaining were scored to determine the number of test and reference probe hybridization signals. Leukocytes infiltrating the tumor were excluded from analysis. Control hybridizations to normal fibroblast interphase nuclei were done to ascertain that the probes recognized a single copy target and that the hybridization efficiencies of the test and reference probes were similar.

The scoring results were expressed both as the mean number of hybridization signals per cell and as mean level of amplification (= mean of number of signals relative to the number of reference probe signals).

**DNA flow cytometry and steroid receptor analyses.**

DNA flow cytometry was performed from frozen 200  $\mu$ m sections as described by Kallioniemi, *Cytometry* 9: 164-169 (1988). Analysis was carried out using an EPICS C flow cytometer (Coulter Electronics Inc., Hialeah, Florida, USA) and the MultiCycle program (Phoenix Flow Systems, San Diego, California, USA). DNA-index over 1.07 (in over 20 % of cells) was used as a criterion for DNA aneuploidy. In DNA aneuploid histograms, the S-phase was analyzed only from the aneuploid clone. Cell cycle evaluation was successful in 86 % (108/126) of the tumors.

Estrogen (ER) and progesterone (PR) receptors were detected immunohistochemically, from cryostat sections as previously described (17). The staining results were semiquantitatively evaluated and a histoscore greater than or equal to 100 was considered positive for both ER and PR (17).

**Statistical Methods.**

Contingency tables were analyzed with Chi square test for trend. Association between S-phase fraction (continuous variable) and 20q13 amplification was analyzed with Kruskal-Wallis test. Analysis of disease-free survival was

performed using the BMDPIL program and Mautel-Cox test and Cox's proportional hazards model (BMDP2L program) was used in multivariate regression analysis (Dixon *BMDP Statistical Software*. London, Berkeley, Los Angeles: University of California Press, (1981)).

5 **Amplification of 20q13 in primary breast carcinomas by fluorescence *in situ* hybridization.**

The minimal region probe RMC20C001 was used in FISH analysis to assess the 20q13 amplification. FISH was used to analyze both the total number of signals in individual tumor cells and to determine the mean level of amplification (mean copy number with the RMC20C001 probe relative to a 20p-reference probe). In addition, the distribution of the number of signals in the tumor nuclei was also assessed. Tumors were classified into three categories: no, low and high level of amplification. Tumors classified as not amplified showed less than 1.5 than 1.5 fold-copy number of the RMC20C001 as compared to the p-arm control. Those classified as having low-level amplification had 1.5-3-fold average level of amplification. Tumors showing over 3-fold average level of amplification were classified as highly amplified.

20 The highly amplified tumors often showed extensive intratumor heterogeneity with up to 40 signals in individual tumor cells. In highly amplified tumors, the RMC20C001 probe signals were always arranged in clusters by FISH, which indicates location of the amplified DNA sequences in close proximity to one another e.g. in a tandem array. Low level 20q13 amplification was found in 29 of the 132 primary tumors (22 %), whereas nine cases (6.8 %) showed high level amplification. The overall prevalence of increased copy number in 20q13 was thus 25 29 % (38/132).

**Defining the minimal region of amplification.**

30 The average copy number of four probes flanking RMC20C001 was determined in the nine highly amplified tumors. The flanking probes tested were malanocortin-3-receptor (MC3R, FLpter 0.81), phosphoenolpyruvate carboxykinase (PCK, 0.84), RMC20C026 (0.79) and RMC20C030 (0.85). The amplicon size and location varied slightly from one tumor to another but RMC20C001 was the only probe consistently highly amplified in all nine cases.

**Association of 20q13 amplification with pathological and biological features.**

The 20q13 amplification was significantly associated with high histologic grade of the tumors ( $p=0.01$ ). This correlation was seen both in moderately and highly amplified tumors (Table 4). Amplification of 20q13 was also significantly associated with aneuploidy as determined by DNA flow cytometry ( $p=0.01$ , Table 4). The mean cell proliferation activity, measured as the percentage of cells in the S-phase fraction, increased ( $p=0.0085$  by Kruskal-Wallis test) with the level of amplification in tumors with no, low and high levels of amplification (Table 4). No association was found with the age of the patient, primary tumor size, axillary nodal or steroid hormone-receptor status (Table 4).

**Table 4. Clinicopathological correlations of amplification at chromosomal region 20q13 in 132 primary breast cancers.**

	Pathobiologic feature	20q13 amplification status			p-value <sup>1</sup>
		NO Number of patients (%)	LOW LEVEL Number of patients (%)	HIGH LEVEL Number of patients (%)	
5	All primary tumors	94 (71%)	29 (22%)	9 (6.8%)	
10	Age of patients				
	< 50 years	17 (65%)	6 (23%)	3 (12%)	
	≥ 50 years	77 (73%)	23 (22%)	6 (5.7%)	.39
15	Tumor size				
	< 2 cm	33 (79%)	7 (17%)	2 (4.8%)	
	≥ 2 cm	58 (67%)	22 (25%)	7 (8.0%)	.16
20	Nodal status				
	Negative	49 (67%)	19 (26%)	5 (6.8%)	
	Positive	41 (75%)	10 (18%)	4 (7.3%)	.41
25	Histologic grade				
	I - II	72 (76%)	18 (19%)	5 (5.3%)	
	III	16 (52%)	11 (35%)	4 (13%)	.01
30	Estrogen receptor status				
	Negative	30 (67%)	10 (22%)	5 (11%)	
	Positive	59 (72%)	19 (23%)	4 (4.9%)	.42
35	Progesterone receptor status				
	Negative	57 (69%)	20 (24%)	6 (7.2%)	
	Positive	32 (74%)	8 (19%)	3 (7.0%)	.53
40	DNA ploidy				
	Diploid	45 (82%)	8 (14.5%)	2 (3.6%)	.01
	Aneuploid	44 (62%)	20 (28%)	7 (10%)	
	S-phase fraction (%)	mean ± SD	mean ± SD	mean ± SD	.0085 <sup>1</sup>
		9.9 ± 7.2	12.6 ± 6.7	19.0 ± 10.5	

<sup>1</sup> Kruskal-Wallis Test.

#### Relationship between 20q13 amplification and disease-free survival.

Disease-free survival of patients with high-level 20q13 amplification was significantly shorter than for patients with no or only low-level amplification (p=0.04). Disease-free survival of patients with moderately amplified tumors did not differ significantly from that of patients with no amplification. Among the node-negative patients (n=79), high level 20q13 amplification was a highly significant prognostic factor for shorter disease-free survival (p=0.002), even in multivariate

Cox's regression analysis ( $p=0.026$ ) after adjustment for tumor size ER, PR grade, ploidy and S-phase fraction.

**20q13 amplification in metastatic breast tumors.**

Two of 11 metastatic breast tumors had low level and one high level  
5 20q13 amplification. Thus, the overall prevalence (27%) of increased 20q13 copy number in metastatic tumors was a similar to that observed in the primary tumors. Both a primary and a metastatic tumor specimens were available from one of the patients. This 29-year old patient developed a pectoral muscle infiltrating metastasis  
10 eight months after total mastectomy. The patient did not receive adjuvant or radiation therapy after mastectomy. The majority of tumor cells in the primary tumor showed a low level amplification, although individual tumor cells (less than 5 % of total) contained 8-20 copies per cell by FISH.. In contrast, all tumor cells from metastasis showed high level 20q13 amplification (12-50 copies per cell). The absolute copy number of the reference probe remained the same suggesting that high level  
15 amplification was not a result of an increased degree of aneuploidy.

**Diagnostic and Prognostic Value of the 20q13 Amplification.**

The present findings suggest that the newly-discovered 20q13 amplification may be an important component of the genetic progression pathway of certain breast carcinomas. Specifically, the foregoing experiments establish that: 1)  
20 High-level 20q13 amplification, detected in 7% of the tumors, was significantly associated with decreased disease-free survival in node-negative breast cancer patients, as well as with indirect indicators of high-malignant potential, such as high grade and S-phase fraction. 2) Low-level amplification, which was much more common, was also associated with clinicopathological features of aggressive tumors, but was not prognostically significant. 3) The level of amplification of RMC20C001 remains higher than amplification of nearby candidate genes and loci indicating that a novel oncogene is located in the vicinity of RMC20C001.

High-level 20q13 amplification was defined by the presence of more than 3-fold higher copy number of the 20q13 amplification is somewhat lower than the amplification frequencies reported for some of the other breast cancer oncogenes, such as ERBB2 (17q12) and Cyclin-D (11q13) (Borg *et al.*, *Oncogene*, 6: 137-143 (1991), Van de Vijver *et al.* *Adv. Canc. Res.*, 61: 25-56 (1993)). However, similar

to what has been previously found with these other oncogenes (Swab, *et al.*, *Genes Chrom. Canc.*, 1: 181-193 (1990), Borg *et al.*, *supra*.), high-level 20q13 amplification was more common in tumors with high grade or high S-phase fraction and in cases with poor prognosis. Although only a small number of node-negative patients was analyzed, our results suggest that 20q13 amplification might have independent role as a prognostic indicator. Studies to address this question in large patient materials are warranted. Moreover, based on these survival correlations, the currently unknown, putative oncogene amplified in this locus may confer an aggressive phenotype. Thus, cloning of this gene is an important goal. Based on the association of amplification with highly proliferative tumors one could hypothesize a role for this gene in the growth regulation of the cell.

The role of the low-level 20q13 amplification as a significant event in tumor progression appears less clear. Low-level amplification was defined as 1.5-3-fold increased average copy number of the 20q13 probe relative to the p-arm control. In addition, these tumors characteristically lacked individual tumor cells with very high copy numbers, and showed a scattered, not clustered, appearance of the signals. Accurate distinction between high and low level 20q13 amplification can only be reliably done by FISH, whereas Southern and slot blot analyses are likely to be able to detect only high-level amplification, in which substantial elevation of the average gene copy number takes place. This distinction is important, because only the high amplified tumors were associated with adverse clinical outcome. Tumors with low-level 20q13 amplification appeared to have many clinicopathological features that were in between of those found for tumors with no and those with high level amplification. For example, the average tumor S-phase fraction was lowest in the non-amplified tumors and highest in the highly amplified tumors. One possibility is that low-level amplification precedes the development of high level amplification. This has been shown to be the case, e.g., in the development of drug resistance-gene amplification *in vitro* (Stark, *Adv. Canc. Res.*, 61: 87-113 (1993)). Evidence supporting this hypothesis was found in one of our patients, whose local metastasis contained a much higher level of 20q13 amplification than the primary tumor operated 8 months earlier.

Finally, our previous paper reported a 1.5 Mb critical region defined by RMC20C001 probe and exclusion of candidate genes in breast cancer cell lines and in a limited number of primary breast tumors. Results of the present study confirm these findings by showing conclusively in a larger set of primary tumors that the critical region of amplification is indeed defined by this probe.

The present data thus suggest that the high-level 20q13 amplification may be a significant step in the progression of certain breast tumors to a more malignant phenotype. The clinical and prognostic implications of 20q13 amplification are striking and location of the minimal region of amplification at 20q13 has now been defined.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference for all purposes.

#### Discussion of the Accompanying Sequence Listing

SEQ ID NOS: 1-10 and 12-13 provide nucleic acid sequences. In each case, the information is presented as a DNA sequence. One of skill will readily understand that the sequence also describes the corresponding RNA (*i.e.*, by substitution of the T residues with U residues) and a variety of conservatively modified variations thereof. The complementary sequence is fully described by comparison to the existing sequence, *i.e.*, the complementary sequence is obtained by using standard base pairing rules for DNA (*e.g.*, A to T, C to G). In addition, the nucleic acid sequence provides the corresponding amino acid sequence by translating the given DNA sequence using the genetic code.

For SEQ ID NO 11, the information is presented as a polypeptide sequence. One of skill will readily understand that the sequence also describes all of the corresponding RNA and DNA sequences which encode the polypeptide, by conversion of the amino acid sequence into the corresponding nucleotide sequence using the genetic code, by alternately assigning each possible codon in each possible

codon position. Similarly, each nucleic acid sequence which is provided also inherently provides all of the nucleic acids which encode the same protein, since one of skill simply translates a selected nucleic acid into a protein and then uses the genetic code to reverse translate all possible nucleic acids from the amino acid sequence.

The sequences also provide a variety of conservatively modified variations by substituting appropriate residues with the exemplar conservative amino acid substitutions provided, e.g., in the Definitions section above.

## SEQUENCE LISTING

## SEQ. ID. No. 1

3bf4 3000 bp

5 CCGCCGGCGGGCGCTGGCTGCACTCAGGCCGGACGGCTAGCGGCCGCCCATGTCCCACCAAGACCGGCAT  
 CCAAGCAAGTGAAGATGTTAAAGAGATCTTGCAGAGCCAGAAATGAAAGTACAGACTCTGAAAATATCTATTGAA  
 ATGAGCAACTTGTGATTGGATCATATAGTCAGCCTCAGATTCTGGATAAGGATTATGATTCCCTTGTTTACCCCTG  
 TTGGAGGACAAACACCAGCTATAATTATTACAGGTTAGATTCTCAGAATGCCAGGGATATGAATGGATATTCACTGC  
 ATGGTCTCCAGATCATTCATGTTCTCAAAAAATGTTGATGCAGCAACAAGAGCAACTCTGAAGAAGGAATTGGAC  
 GTGGCCACATTAAGAGATGAAAGTATTGGAACAGTAAGGAAGATGATTCATTACATGGATAAAAAAATACTTGCTGCA  
 10 CAATCTCCCTGCCCCACTGACTGCAGTGAGGAAGAACTACAGACAGATTTAAATCAATGAGGATACAGACTGACGTGGG  
 TGTGGACACTAACATCAAACACTACAAGGGTAGCCATTCCCAATTCTCGAGAAGCCCTTGGAAAATTGAA  
 ATAATAGACAGCTCAACTATGTGCACTGGAAATAGATAAAAAAATGAAATTATTTGGCCAACACAACAAATACA  
 GAACTGAAAGATTGCGAAAGAGGATTCCAAAGGATTAGCTCGTTACCAATTCTGATAAACATTCCTGAGG  
 15 AGACTATTAGTCCATAGTTTATTATTCAATGCTGGATACACATGCAGTATAAGAGAGGGATGCTGATTCTA  
 GCTGCAAGAGCCCTGCTGAGAAAATTGAGAAAGACAACATCAAATGGATGTAATTAGAAAGATCGAGATAGACAATGGG  
 GATGAGTTGACTCCAGACTTCTTATGAAAGAAGTACATCCAGCAGCATGCACACAAGCAAGGTTTGCAAAACAAA  
 AGGTCTGCAGGAAAAAGAGAATTGAGACTAATTAGGGGCCAGGGAAACTGAAGCTACTGATTAAAGTCATC  
 ACATTAACATTGTAATACTAGTTTTAAAGTCAGCTTAACTGAGGAGAACTGAAATTCAATTCCATGTTGATA  
 20 AGTAGGGAAAAAAATTGACTTTTGAAAAATGCACTTTTCACTCTGTTGTTTAAATTGTTAAGGACT  
 CATGATTCTATTGAGTTAAAGCTGAGGTTCAACATAATGTTTATTGTCACACTGTTTCAAGCGTTG  
 ATTCCACACTTCAAAACTTCTTAAATGAAAGTACAGTTGGGCCAGTTCTAGAAAAGTCTGATGCTCAAGGGTAAACTT  
 ACTACTTCTGAGGACAGAAAGACCTAAAAATATTCATATTACTAAATGAAATATGTTAAGGACAGGCTAGAGTATT  
 TCTAAGCTGAAACTTAGTGCCCTGGAAAAGCCCAAGTTGCTTACTCCGAGTAGCTGTTGAGCTGCTGCTAGACTGT  
 25 AGGATCATGCTGCAACTTTAGAAAATAGTGTGTTTATTGAGCAGTCTTATATTGAGCAGTCTTATTTGACTTTTAAATAGCATTAA  
 AAATTGAGATCAGCTACTGAAACTTTAAGGGTACCGAGATTTCTACTGCTGAGGCCAAACATTATGTTAGGAAATTAAATTAA  
 ACTTTAACAGCCTTAGAAATTATCTTCAATTGCTGAGGAGGTTCTCCAAACATACATTATGTTG  
 TATCATTCAAAGGAAACAAAAATGTTGAGTTTTAAAGGTTCTGAGGAGGTTCTCCAAACATACATTATGTTG  
 30 CAAATTGTTCTTATCAGTTCCAGGAAAATCTAGTATTCAAAACACATTAACTGTTTCAATGTTTCAATTGTTG  
 TAGATAAACACCTTAAATCTAGTAAATGTTGAGGCTAAACTGTTTCAATGTTTCAATTGTTG  
 TTACAACCTAGAGAGATTGAGCCTGAAACTTTGAGGCTGAAACTTGTGAGGCTTCAATTGTTG  
 GTTAAACCTGCTGCAAGAGCCATAACTTTGAGGCTTCAATTGTTGAGGCTTCAATTGTTG  
 CTAAACTTTATGTCATAAATCAGTTATGGAAATGCACTTCAATTGTTGAGGCTTCAATTGTTG  
 TAAGGTAATATATGCACTTTCAAGAAAATTGTTGTTGAGTAAAGCATTAGAATTAATTGTTG  
 35 TAAAGATAATTAGAGATTGTTGGGTTGTTGAGTAAAGCTTCAATTGAGGAGCTAGAACACTTAAATGTTAATCT  
 ACAATAATGTTGCACTTTGTTACTAAAGATAATGTTGTTCAATTGAGGAGCTAGAAGACACTTGTCAATTGTTG  
 TATTGCAATTAGAATCTGGCAGGACCATAGCTGAGCTGTTATTGAGGAGCTAGTCAATTGTTG  
 AAATAATTAGAAGTGAATGTTTCTGTTGACCATCTATGTCATTAACTCTAAATTCCACTACACTACATTAAAGTAA  
 ATGGACATTCCAGAATATAGATGTGATTAGTCTAAACTAAATTATTAACCAATGTTGCTGAAATTCACTGTTG  
 40 CATTGTTATAGAGTATAACTCATGCTTACAGTATGTTTGTGAGGAGCTACACTAGATGGTAATTACAT  
 CCAGTAAATTATAGCAGTGAAGAATTACATGCTTCTGGTGACATTATAAGTGCAATTATCACAATTAAAAA  
 TTTTTCTCTTAAAAACAAAGAAAAAA

## SEQ. ID. No. 2

1b11 723 bp

45 TGGAAGCTGTCATGGTACCGTCTCTAACGTTGGACTCTAAAGAAAATGATTATTCTGGTTCTAGACAGGCCAAATGT  
 AATTCACTACGGCAGATTAAGAGGTGGCTTACTAGATTGATTGGTATTGAGCATGCTCTGAATGACAGTCCCC  
 AAAAGGACCTCTTATCCGTTCTCCCTGGGGAGGGCTTTGCCACTTCCATGTCATGTGGCAGTTGAGCTTGGAA  
 ATTGGTGCCTGTCACACATAAGCATTACTCTCAGAAGATGTCCTGTTGAGAAAATGTCATAGATTCAAACACTGAGCT  
 ACTATGTTGAGGGGGCAGCAAGGACCCACTTTGTTAAAGCTTGTGTTTCTGAGGAGCTTCAATTGTTG  
 50 TACCTGGCAAAATAATCCAGGTGGTGTGAGTCACCGTAGAGGTTAAAGCTCAAGGAAGTGAATCAGCTTACAA  
 ACAGTGGACCTCAACGAGAGAGATGCTGCACTGAGCAGGAACTCAAAAGAGAAGAAAGCAAAACCAAGAAC  
 CTCTCTGATGRGTTCTCAGACAAATGGTAAGCCCTTACTTCCAGTAATAGAAACCTAAGATAACCTAGAGGGCTTT  
 GGGACAATGGGCTCATGCCACAGGTAGTAGGAGACATAATTGAGCTGGTGTATGGAATGTGAATGGAATATGGATT  
 GCG

55

## SEQ. ID. No. 3

cc49 1507 bp

60 GCAGGTTGCTGGGATTGACTTCTTGCTCAATTGAAACACTCATCAATGGAGACAAAGAGCACTAATGCTTGTGCTGAT  
 TCATATTGAAATCGAGGCATTGGGAAACCTGTTATGCTTGTGAAAGAACCAGTGACACCATCACTGAGCTTCTA  
 AAAGTTGAGAGATTAGAGGACTATACACTTTCTTGAACCTTAAATTGCTCTGTTTGGAAACCCAGG  
 ACTTTAGAGGGTGTGAGTCAGGTTCTACAGTGGCTTAACTCAACTCCAGAAATTGCCAACGAAACTTGGAGATTATA  
 TGCAATGCAAGTGAAGGAAACATGCCAACTCAATCCCTTAAATGTCATGGATGCCAAGAGTGATTGGCAGCTCTC  
 TTGCACTGCAAGTGAAGGAAACATGCCAACTCAATCCCTTAAATGTCATGGATGCCAAGAGTGATTGGCAGCTCTC  
 ATGTCATGCCAACGAGGAAATGCCCCTGGATTGCAATTGAGGAGCTACACAAAGAAAAAA  
 65 TAAACATGTCATAATGCAACACCGGCCATACCCCTCTGTAACCAGCAGTTCTGGGTTGAAGCAGAGTATCTCAGTCCGC

TTGATAAAAGTCAAGTCCGAACAGAACCTCCCAAGGAAAAGAATTGCAAGGAAAATGAATTTCAGTGAGGTATGTGGG  
 CAGACATTAGACTGCCTTTGATGTTGAGATCCACATGGAACACACAAAAGATTCTTCACTTACGGGTGTAACATGTG  
 CGGAAGAAGATTCAAGGAGCCTTGGGTTCTTAAACATCAGCGGACRCATATGCAAACTCGGGGCCAGAAGCAAA  
 5 TGCAGCAAGGCTTGGAGAGTAGTCAGCACGCAACGATCAACGAGGTCGTCAGGTCAGCAGGGGCCAGAAGCAAA  
 TGCAAAATCTCCATGGCTTGTGGCTCTTAAATTCAAAATAAGAAAAGTCTAATTGAGCAACGCAAGGTGCAACCAAAA  
 AACTGCTTCGGTACCGCAGCGCAGACAGACTCTCCACAAGGAGGAATGCCGCTCGAGGGGAGGACTTCCTGCAGT  
 TGTTCACTTGAGACCAAAATCTCACCCCTGAAACGGGAAAAGAAGGCTGTCAGATGCACTCCCTCAGCTGATCCGTTCA  
 10 ACCTTCAGGCTTGGCAKCTGGCTACCAAAGGAAWAGTTGCCATTGCCAAGAAGTGAAGGAATTGGGCAAGAAGGGAG  
 CACCGACAAACGACGATTGAGTTCCGAGAAGGAGCTTGGAGAAACAAAATAAGAACCAATTGTCAGGCCCTCGCAAGAGA  
 AAGAGAAGTCAAAACACTCCCACGGCAAGGCCCTCCGTTGGACGGATCCCAGTACCAAGGAGAAGGCC  
 ACTCACTGCTCCGAGTGCAGCAAAAGCTTCAAGAACCTACCAACAGCTGGTCTGCACTCCAGGGTCC

## SEQ. ID. No. 4

cc43 2605 bp

15 CAAGCTCGAAATTAAACCTCACTAAAGGAAACAAAAGCTGGAGCTCCACCGGGTGGCGGCCCTAGAACATAGTGGAT  
 CCCCCGGGCTGCAGGAATTGGCACCGCTGGCTACTACGATGGCGATGAGTTTCGAGTGGCCGTGGCAGTATCGCTTC  
 CCACCCCTCTTACGTTACACCGAAATGTGGACACTCGGCAAGCAGCTGGCCGCTGGTCTGGCTGGTCTGTCTT  
 CTGGCCGCTGCACAAACAGTCCAGCATGAGCTGGAGATGGAAGCTCAGGAGAGGCCGCTTCAACACGTCAGCTACAGC  
 GAAAGCTTCTGTGGAGTCGATCAGATTGATTAGAGGAAGTGGAGGAAGAAGGGAACTTCGAGTGGTTGGATAAGAGC  
 20 AAGTCCAGCTTCTGTGATCATGTGGCCGAGGCAAGAAATGGGAAAAGGAACTCATCTATCAGTGGTTCCAGGAGTGGCCA  
 GAACAACACTCGCTTTCACCCCTGATGAACTGACTAATGGGAAAGAACAGAGGATGAGGAGTTCCAGGGCTGGATGAA  
 CCACTTACTCGGCTTCTGCAGGCCCTACAGCAGGAGCACAAAGGCCGAGATCATCTGTCAAGCGATGGCCGAGGCC  
 AAGTTCTCTAGCAGGGACCTGTCCTCCCTTACTCTTACTCTCCACCTTCTCAGGGCTTCAAAAGGAGACAGACCCAG  
 25 TGTCCCCCCTAAAGACTGGATCTGTGACTCCACAGACTCAAAAGGACTCCAGTCCCTGAGGCTGAGCACAGGGAGAAAATATGTGCTTCTTCTGCC  
 TCTCACACCCCATATGTCGTGCCCCGGATAGGGTGAGGCTGAGCACAGGGAGAAAATATGTGCTTCTTCTGCC  
 CCTCTTCTCCATCTAGTGTGCCCCGGATCTGAGGCTGAGCACAGGGAGAAAATATGTGCTTCTTCTGCC  
 TACACACATGCGCTGCAAGCACATGCTTCTGCTCTCCCTCCACCCCTTAAAGCTGCTGCTGCTCCCTCC  
 30 TGGTGCTGGATCTCTCCATGGGGATGGGGAAAGGCCCTGGCTGAGGCAACCCGATTATGATATTAAATCTCA  
 CGGGCTGGCAGTGAGGGTGTGGGCCCCACCCGATTATGATATTAAATCTCA  
 CTGAGACTTAGTCTCTCTGAGAACACTGTCAGTTTTTTTTTTTTTTTTTTTT  
 GCTTTAAGGATTATTTATGTTCTCTTACAGTGTCACTTTCTACTAAACTACTTCCAGTCAGAAGCC  
 CAGAGGGAAAAAAAGACCATGAAATCTCTCTCCAGATTAAAGTACACATTGGAAAAGCATTGGAAAACCTTT  
 35 CTGAAAAAAAGTGAATGAAACTCCAAACACATGCCATTGTTGAGTTGCTCATGAAATTTGTTAAAACCTGTTCT  
 AGATAAAAGAACAGTCTCAAGTTTGTACAGCTACATAGTACAAGGGTCCCTATGATGTTCTGTAGGAGCAA  
 ATAAATGTAATTTTCTGAGTTCTGGTTATAACTCTCTGCACTCAGAGTTGACTGATEATTAAACCTACTCATGCAACA  
 GAGAAATTAAACACTCATATTTTTATGAGAACAAACTATTGGAAATTCTATCTATTGGAGACACAAATATGC  
 40 TGGACTAAAGCAATTATTATTCTCAATGTCTGTGCAACCTCAATGACTTAGAATGCTTATTTGGCTATATTTGCC  
 TATGCCCTCAACCACACTGGCTTCTTTAGTCTTGTGAAACAAGCC  
 AACTGCTCTGCCTCAGGACCAAGATAATTGGGACTTCTTAAAGAATTCTTATCTGGGTAAC  
 TAGTTTATCAACACTTCAGATCTGCCGTTAAACACTCTTATAGAACGCTGTCACTGTCCTCTTCTCC  
 CATACTCACAGCACACATGTAGACTAGATTAGAACCTCTGTTTCTCATACTTTCTCTATCATGCTTCC  
 45 CATTATAATTCTTATATGTTGAGTGTGAAATGTCGCCCCAACGTCAGTTCTCTCACTAAACTATAAAACTCCGTAAGCTGG  
 GATCTCTCCAAATTGATCACCACACTAGTACAGTAGGAACACAGTCAAGGTTCTGTTGGAATGAATGAATG  
 AATTGTTCTGAGTAAAGTCTGGGGAAACCCAGGTGAGAAGAGGCTAGAAAAGCAGGTCGATCCAAGGGTAGATGACT  
 TAGTGTACTCAAGAAAAGGGTAGCCTGAAAATTAAAGGTTCAATTAGTCAGAACATAGTCAGAACATGGGAAAGACAAG  
 AGTGTGCTCGTGTGGCAATTGCAATCAAGCTATGATACCGTCGACCTCGAGGGGGGGGGGGTACCCAAATTGCC  
 50 41.1 1288 bp  
 AAGGGCAGGGAGAAGGAGAAACCCAGCCCCCTGGAGCCACATGCTCTGAGCAATGGGTGGCCCTCGCCAACCAAGC  
 CCCGGCCCTGCCATGCAACCAACTCAGGCCCTGCACTGGCTCTGAAACAACTCACTGGGCAAGGCCAGGGGCC  
 TGGCTCTGAGTCTGCTCCACACCGCAAGTCAGCAGCGTGTCCAGGCTACCTGTTGAGAACAGCGATGAGCCATTGA  
 55 CCTGACCAAGTCCAAAAGCAAGAACGCCAGTCTGCAAGCACAATCTGTTGATGCTCCACCTCAGAACAGCAGCTCTGT  
 CTGACATGCCGACATGGCAAGTCTCTCCAAAGCCACCCACCCAAAGCCAGCCTCTCTCCAGGGTCCCCCATG  
 AAAGCTGGAAATGGATGTCAGGCGCTTGTGAGGATGTCAGTCAGTCAACTTGTCAAAAGAAAAGGCC  
 CAAACTGGAAATCTCTGAGCATCTCTGATTCTACAAGGCCAGTTGCTCTGAGCCTCTCCAGACATCAGAGGGCAAATACC  
 TGCTGTCGATCTGGGCCCACAAGAGCGTATGCAAAATCTCTAAGTTACGGGACTCTCAATGAGCACTATCAGTC  
 60 CTGGGCAACGTCAGTACCGCTTGTGAGGAAAAGGGGGGGGAAATTCTGAAAACATGGACAAGGCCACCCCATCT  
 TTATTGCACTGACTGTGCTCTCCAGTTGCAAGAACCTCTCTACCTCATCTGACTTGAAGTCTACCTGACCTGGGTTCC  
 TGAAGGACATGACCCGCTTGTGAGGACAGAGACTCTAAATTCAAGTGAAGTGTGCTGTCGGACATTGTGAGCAA  
 ACATGCGGTTAAACTCACCTAACGAAAAGCAGCACAGCAACTCACCTGAGGGGGGGGGGGTACCCAAATTGCC  
 65 AAGAAATGCTCTGCAGGACGAATGCCATTGTTCACTTCCACCTGGATCCCTCACACTGAAACCCCTTCTGTC  
 CCATCCTGCTCTGACATTGAACTCATGAACTCCCTCTGACACCCCTGGCTCTGAGAACAGACTGCC  
 AAAATTTC

SEO. ID. No. 6

**GCAP 2820 bp**

ATCCTAAGACGCACAGCTGGGAAGCCAGCACTGGGGAGTGGTGTGAGGGATGTTGGTCAGGGTCACTGGGGTGAAGGTGGAGC  
TTTCAGGGTCTCCCGTCAATGCAGCTGAGTTTCTTGGCAGGGAAATTACCACTGAAAGAACGGCTCGCCGGCAGAGACT  
ACAAACTGAGCAAGGCCAGCTCAGCACACCCGGAGGAGTGTGCTGCACTCTGAGTCTCTCCCTGGTGGATGAGAAATGGAGATG  
GTAAGAGGGCAGAGATGGGGAGTGTGCTGCACTCTGAGTCTCTGGCAGGGAGTGTGCTGCACTCTGGCAGCTGGGCAAGGCC  
CTCCACCTTCAACCTGGGGTCTCATCTGTGAGAAGGCTGTGGAGAAGAGATGTGCTGAACTAACAAAGGGACTCATGAG  
CACCGTGTGAGGAGTCAAAAGTCTCAAGGAGTTGCTGATGGAGGCCAGGCAGAAAGAGAAATAGGAAC  
TTTGGAGTCAGGAGGGAGTGATAATTGAGCTTCTGCTCTAGTCTCAATTTCCTCATCTGGAAAATGGGGATAAATAAAT  
AGTGGTTAGAGGAATGAATAGGATAATGTGTTAAAGAGCAGGCAGGGTAGACCTCCATTAGGCTGCTGGGCTTTC  
CTCCCTGTAGCCCAGGCCAGCTCAGGGTATGTGGGAGAGAGCTGGCTGGAAATACACACTTGTGAGGCCCTCCAGCTC  
TCTCAGCTCCACCCAGCATTCCGTGGTACCATGCGAAAAGTAAACTTCATTCATCAGCAAAGAAAGCCCCCTDIAAG  
GTGGCAGGAGACTCTTGGAGATTAGACACCTGACAAGCCGCAAGCTTGGAGGTCTGAGACTCAGGAGTAGTGGGCTAAG  
ACGTGTAGGCGCATTCTGGGAGGGTCTCTCTCTGGGAGGGTCTCTGAGCTCAGGAGTTCTGAGGTCAGGAGTAGTGGGAGTGC  
TCTCCTCCCTGTCTAGGCCAGCTGTCTCTGAGCTTCTGAGGTTCTGAGGTCAGGAGTAGTGGGAGTGAAGATG  
TGGAGATGGACATGAATCCCAGCAGCTGGCTCGCTCAGCAGAGACGGAAAAGTGCCTGTTCTGAGGAGTAGTGGGGCCCC  
TCCACGACTCCAGGCTCACCCAGGTTTCCAGGGTAGTAGGGAGGGTCCCCCTGGCTCAGGCTGTCATGCCCACTCTTCCCC  
TGGTGTGACTTCTGGCACCCCCCTGTGCAAGGCTGAGTGGGGAGGGCTGAGTGGGGAAAGGGCTGCTGGGTTTGAAGTGGGCAACAGG  
GCATAGTCCATTGGAGGAGTCCCTGGGATGGTGAAGGGAAATTAGCTTACTTTCTGTTAGGCCCTCTGGGAGGAC  
TGTGCCCTGGCTGGGTGGTTGTGGGGCTCCACAGTTCTGGGTGTGCTAGTTGGAGGCAAGAGCCAACCTGAGGGGTGA  
GGGTTCCACAGACCAAATCAAGAAATGAACTGGAGGGTGGGAGGGTGGTGAAGTGAAGAGAAA  
AGGCAGGAGTTGGGGGGCAGGGGGCAGCAAAATTCTATAACTTCCGGGATGTCAGGGGGCGAGGCTGAGGCT  
AGGAGATGCGAGAACCTCTGGCTAACACGGGGTGAAACCCGGTCTCTACTAAAAATACAAAAATCAGCCGGGTGAGGTG  
GGGGGGCCTGTAGTCCAGCTACTCAGGAGGTGAGGCAAGAGAAATGGCGTGAACCCAGGGGGCGAGCTACAGTGA  
GCCAGGAGATTGCCACTGCACCTCCAGGCTGGAGGACAGTGAGACTCCGTCTCAAAAAAAAAAAAAGAAAAGAAAAG  
GCAGGAGTTGGGGGGCAGGGGGCAGCAAAATTCTATAACTTCCGGGATGTCAGGGGGCGTTCATGGGGAGGACCTCTG  
GCCTCCCTCTCCCCAAGGCATCTCACCAGGGTGTCAACAGGAAAATGGCAGCAAATACGCTGCAAGGGCTGCTGGTCTT  
CTGCTCTTGTAAAGGGTCACTGTGACTTAAAGGGAGTGTGCTCTGGGAGGCTGCTGGGACCCCTGTGCTGCCA  
ACCCACCAACTCCCCAACATACTCTCTCTTCCATCCATACTCCCCAGTATGGACCTTCCACAAACTCCCAGCCATAAGCTG  
AATGGTTCTTAAAGGATGGAGAAAATCTCTGTGCTGTGAGAAGATGGGGAGCTGTTGACTGGGATTGTGGG  
TGGGGCTGGCTTCTAATGCTGTGACCCAGACAGCCACTCTCTCCCTAACCTTGGTATGTCTGGCAGCACAGT  
GAGCAGGCTGGAGCTAGGCAGACAGTTGGATTATGTGTTTAAAGTGTGGAAATTATTTTGTATATAAAACTCTTAT  
GTGTAACCCCCAATATAGAAACTAGATTTAAAGGGAGTCTCTGGGAGGCTGAGTACCCCTGTGGAACCTGG  
GCACCTCTGAAAAAGCAACTGAAAACCAGTGCCCTGGGACTCTGTTACTCTATAAGACAGTTAAAGTGAAGACTGG  
AAAAACATGGCTTACCTGAAATAGATGGGTTTATGTGTTAATAGAAATAAAACCTTAAACCTTAAACCTGTGAGAC  
TTTACAGGGTGTATACTGTGAGTGTGCTATATAAAATTCCCTTGTGACATCAATTAGGTTAAAGGATTTAGG  
AAAAATTTGGCCAGGTGCAAGCAGCTCACACCTGCAATTCCAGGACTTTGGGAGGGCCAGGGGGGGTGGATCACCTGAGGT  
CAGGGGGTCAAGACCAAGGCC

SEQ. ID. No. 7

1b4 1205 bp

GGCGCGCTGAGTCGCCGGCCCCCCCCAGTCACGTGACCGCTGACTCGGGCGTCTCCACTATCGCTTACCTACCTCCCTCTGC  
AGGAACCCGGCGATAATGGCTGCCGCTGTGCCCGCGCCGCAATTCTCTCCCGCTGCTTCCCTCTCTCTGGGCTTCTCTGC  
TCCCTCCGCTCCGCATGGCGGCAGCGGCTGCAACACCAAGGCGCCCTTCCCTGGATACGGTCACTTTCTACAAAGTC  
TTCCCAAAGCAAGTTCGTTGGTGAAGTTCGACACCCAGTACCCCTACGGTGAAGCAGGATGAGTTCAAGGCTTC  
TGAAAATCGGGCTTCCAGCGTATCTCTGGCCAGGGTGGGATCTCAGAATTATGTGACAAGCTGAACATGGACCT  
GAGTGAGAAAATACAAGCTGGACAAGAGAGCTAACCCATCTCTACCTCTTCCGGATGGGACTTTGAGAACCCAGTCCC  
ATACACTGGGGCAGTTAGGTGGAGCCATCCAGGGCTGGCTGAAGGGGCAAGGGGCTACCTTAGGTATGCCCTGGCTTG  
CCTGTATACGACGCCCTGGCCCCGGAGTTCATCAGGGCTCTGGTGTGGAGGCCAGGCCCTCTTAAGCAGGGGCAA  
GATAACCTCTCAAGTGTGAAGGAGACTCAGAAGAGTGGCCGAGAAATACCTGAAGATCATGGGAAAGATCTTAGACCAA  
GGGGAGCACCTCCAGCATCAGAGATGACACGGATGCCAGGCTGATTGAGAAGAACAGATGAGTGACGCCAGAGGAG  
AGCTCCAGAAGAGCTTAAACATCTGACTGCCCTCCAGAAGAAGGGGGCCAGAAAGAGGAGCTGTTAAAGGCTGTCTG  
TGATTTCTCAGGGTTTGGGGTAGGGAGGGAGAAGTTAACCTGCTGGCTGTGANTCCTTGTGGAAATAAAGGGGGY  
MSKGAAAAAGWGWTACTAACCCAGATTCTGAGGCCCTGAGTATGCCCTGGACATTGATGCTAACATGACCATGCTGGGA  
TGTCTCTAGCTGGCTGGGGATAGCTGGAGGACTTACTCAGGTGGCTGGTGAATAGACACCTCAGAAGGAATGAGTGCTA  
TAGAGAGGAGAGAGGGAGTGTACTGCCAGGTCTTGAACAGATGTAATTCTCATTCATTAAAGTTCAAGTGTGTTGGTTA  
AGTGG

SEQ. ID. No. 8

20sa7 456 bp

**D2S13** 450 bp  
GAAATCAGAAGTTAAATGACACAATTAAATATATTGATATCTCACACCGGAGNTCTCTTCAAAACATAAGGAGTTA  
GAAATTACAAGTAGGCATATGCTTCCTATATTCAAGATAATTCTTTCGATTAAATTAAATTCCAGATAGAGAGAAGTAATT  
TTTCGGAAAAAGAAATGATAGCTATATTAAAGCAGATAATCATACAAATTACCATGTAGAGACATAAGCAATTTTGGCAT

CATTCGTCCGCTCACTAGGGCGTGTTCCCTCTGGTAGGCCCTTGGAGAGTACCATCTAAGATGGAGGAATGCCTG  
TGGGAAGGGCGGGATGGAGGTGGCTTCTACGCTGAACCCCACACAGGAATCTGCAGCCCACACAGCTGCCCTGCGC  
CGCCTCCATGTGATCATCTGGCAATGAAGTGAATTGTCCTATTTCNGGGGT

5

## SEQ. ID. No. 9

## Genomic Sequence Encoding ZABC1

CCATCATATTCCTTATTTTTGGCGGAGAGGGAGACTTGCTCTGTTGCCAGGCTGGACCAGTGGTGCAT  
CT  
10 TGGCTCACTGCAACCTCCACCTCTGGGTCAAGTGATTCCAAATAGCTGGATTACAGGTGTATTACCAT  
GC  
CCAGCTAATTTTGATTTAGCAGATAAGGGGTTCACCATGTTGCCAGGCTGGCTCCAACCTGGCT  
CA  
15 TGATCCACCCACTCGGCTCCAAAGCATTGGAGTATAGGTGTGAGCCACTATACCGTCTCACATCAT  
AT  
TTCTAATCCGAGACTGTAGAGCTGGTGTCTTTCTAAAGGATGTCAGTAGAGAAGTGGAGTTCCCCAAA  
TT  
ACAGTTCACGTATTAGTCAAGTTCTAAACAGTAATAATGTTGAGAGCTGACATAGGGACTAACTTGGTT  
TT  
20 TTTTTTTTTTTTTCAAAATTCTCACTGAACTTGATTTGCTAAATAAGGACATTTAAAAAAACAAAAA  
AACTCCACTATTGCCATTGCCACTATTGATTTTAAAAATAAGCGTATTAGCATCTAAAGTAGGAAGG  
A  
CCTCAAATAATGAGTCTTGTCTGGCCAGGAAACAGCGTGTCAAGATTGATAACTGTTTCTAGGG  
TA  
25 TGTGCTGTTATTCACTTAAACCTTGCCTGGGACGCTAGCATTCACTAAATACTTGTGAATAAGCAAATGAAA  
CT  
TAAGCTCTATGTATAGAACCTAAGTCACCTCACATTGATTAGCAGAGTAATTGAATATTCTTCAATGTG  
T  
30 AGCTCTATCCCCAGAACACAGAACATTGGAACGTGAAAGGCCATCCATAGTTAACCAACTGCGTTAAATAG  
AT  
AAATAGAAAGATGTGGTATGTGGCAGTGACAACCTGAAGGTTGTACTAGAACACTGGGCTCTGGAGTGTCTA  
TTA  
TATCACACCAAGCTGGTACCCAGCCATGTGTTGATCCTCATTGTGATAGCAACAAAGAAAAGACTCAGGAC  
AT  
35 TCTTCCCTTACCTAATCCTGATCTGCACTTATTAGAAAAGCTTAATGTTAAAGATCTAGTTATTCAA  
A  
CTAAAGATAACAAGGAGTATGAGAATTCTATTGAGTGTAAAGGAGGAGATGTTCCCTGGCTCTGAG  
CC  
40 TGAGGCCTTCTTGCTCTTAAGGAAGTAGAGAGAGGGAGGAAAGTAAAGTATGCTTTGTTTTAAGGTTA  
CT  
TTGCTGGAGTAGTTTGATGCCCTTGGTTCTGGTGAATTAACTGACTTAAGTTAAGTAGTTGGGA  
CT  
45 ATTAAAAACATGCCATCCAAATGTTGCCATAAGGAGGGTATTGGCTTAAAGTTAATTCTTCC  
GG  
AGTAAAATTAGCTCTAAACCAAGCAGCAGAGCTAAATAAGTAATTCCACCTGGCCAGTGCATGATGT  
GA  
AAGGTAGATTAAAAAAATGAGAGGGCCATTCTGATGAAAGACTAACGCATGTTGAAACAGCCCTGGAG  
GAT  
50 TTATTTAAATCTATACATTACAAGGAGCTTGTATGTCTTCCCTATTGTTGTTGGACTAGGAAGCC  
C  
CACCCAGTGTGTTGAAGGCAGAAAGCTGTTGAACAGCAAGCTGGATTGAACAGTGGATTGAGGTTCGAA  
TAT  
55 CCAGTGAACCAAAATATCAGGGTCCCCCTGCCAAGATGAGTGACCATCTGAGGTGTTAAGTATTCTGA  
AT  
GGGGATTAGAAAAGTTCTGTATTCTGCTCATTTGTTGACCTCTGTATGTGAAAATCTCAAGGG  
GT  
60 GTTGGCACTTAGATTCTGGATGCAGATTGTTGATATGAAACAAATTAAATTGTTGATACTG  
G  
ATTAAAAATAGTTACTAAAGTCTTAAATTCTCATCTTAAATTTCACAGTCTTATAGTCTTATAGTTAGGG  
AGGCTGTTGATGGCATCCACATGTGCATTAGTGGCATTTAAATGTATTCACTGATTTAACAAATTCTGA  
CC  
65 TAAAACCTGACATTAGATTAAGTCGGTAAAGCACTGATTAAACTGGATTAACTGGATGAAATTCTGATT  
GT  
AATAAGTGTACTGACTGGATAAAATGCCAATGATTAATTAACAAGCACGTTAACAGGATGCCCTATATATTA  
GT  
TAAAAGTGAAGCAATTGAATTAGGTACCTCTGCTGCGTGGAAAAGACCGTATGACTCACCCACACCAGCCT  
TC  
70 TCTTCGCTCTGAGTGTAGCTAACCGTTCTGTTTTCTAGGGTTGGAAATCCCTGTCTCCAGGTTGC  
T

GGGATTGACTTCTGCTCAATTGAAACACTCATTCAATGGAGACAAAGAGAACTAATGCTTTGTGCTGATTCA  
 AT  
 TTGAATCGAGGCATTGGGAACCCCTGTATGCCCTGTTGTGAAAGAACCGAGTACACCATCACTGAGCTTC  
 AA  
 AGTCGAAGAAGTTAGAGGAACTATACTTCTTTGAACCTTATAATAAAATATTGCTCTGGTTTTGGAA  
 C  
 AGGGCTGTTAGAGGGGTGACTGACAAGTCTTACAAGTGGCTTATTCAAACCTCAGAAATTGCCAACGGAA  
 TTT  
 GAGATTATATGCAATCGAAAGTACAGGAAACATGCCACTCAATCCCTTTAATGTACATGGATGGCCAGA  
 10 AGT  
 GATGGCAGCTCTTGGCAGTCCGATGGAGATGGAGGATGCCCTGTCATGAAAGGGACCGCTGTTCTCCA  
 TTC  
 CGAGCTACACAAGAAAAAAATGTATCCAAATCGAGGGGTATATGCCCTGGATTGATGTCAGTTC  
 CCT  
 TCACACATTAGAAGACCTTAATAAACATGTCCTAATGCAACACCGGCCTACCCCTGTGAACCAGCAGTCTT  
 CG  
 GGTGAAGCAGAGTATCTCAGTCCCTTGATAAAAGTCAGTGCAGAACAGAACCTCCAAGGAAAAGAATTGC  
 AAG  
 GAAAATGAATTAGCTGTGAGGTATGTGGCAGACATTAGTCAGTGCCTTGTGAGATCCACATGAGAAC  
 20 AC  
 ACAAAAGATTCTTCACTTACGGGTGTAACATGTGCGGAAGAAGATTCAAGGAGCCTTGGTTCTTAAATCAC  
 AT  
 GCGGACACATAATGGCAAATCGGGGCCAGAAGCAAACCTGCAGCAAGGCTTGGAGAGTAGTCAGCAACGAT  
 CAAC  
 25 GAGGTCTCCAGGTGCACGCCAGAGAGCATCTCCTCTCCTACAAAATCTGCATGGTTGTGCCCTCTTATT  
 TC  
 CAAATAAAAGAAAGTCTAATTGAGCACCGCAAGGTGCACACCAAAAAACTGCTTCGGTACCGCAGCGCGCA  
 GAC  
 AGACTCTCCACAAGGAGGAATGCCCTCTCGAGGGAGGACTTCTGCAGTTGTTCAACTTGAGACCAAAATCT  
 30 CAC  
 CCTGAAACGGGAAGAACCTGTCAGATGCATCCCTCAGCTCGATCCGTTCACACCTCCAGGCTTGGCAGC  
 TGG  
 CTACCAAAGGAAAAGTGGCATTGCCAAGAAGTGAAGGAATGGCAAGAAGGGAGCACCACAGGAC  
 ATTIC  
 35 GAGTTCCGAGAAGGAGCTTGGAGAAACAATAAGGGCAGTTGTGCAGGCCCTCTCGAAGAGAAAGAGAAGTG  
 CAAA  
 CACTCCCACGGCAAGGCCCTCCGTGGACGGATCCAAGTTACCCAGTAGCAAGGAGAAGGCCACTCACT  
 GCT  
 40 CCGAGTGGGAAAGCTTCAAGAACCTACCAACAGCTGGTCTTGCACCTCAGGGTCCACAAGAAGGACGGAG  
 GGC  
 CGGGCGGAGTGGCCCACCATGTCGGACGGAGGAGCCGGAGCTTCTGCACCTCGCCGCCCC  
 TCTG  
 GATGAAAATGGAGCCGTGGATCGAGGGAGGGTGGTTCTGAAGACGGATCTGAGGATGGCTCCCGAAGGA  
 ATCC  
 45 ATCTGGTAAGCTGCCCTGTCTCCGCTGCTGTTCCGCTGTGTCGTCGTCCTCCCGTCTCCCCCTCTC  
 TA  
 TTCCCATCTCAGACAACGCTGCCAGGAATGGGTTGGAGAGCCAGACTCAAGTCCAGGCTTTGGTA  
 TCA  
 CTCTGTGAAGTCATTTAACCTCTAGGGCTTAATTTCTCATTTCTGAATAACAGGGTTGAGTTAAGAGGT  
 T  
 50 CCTTGTGAAATATATATATATTTAAACGTGATCGTTGCTCACAAACACACTTTAAAAAAAT  
 A  
 ACTTGTGCATCCAGCCAAATGCACTGCTTCTAAGTGGGCGATTGCTCCAAATCAGTATCTGGCAATGTC  
 TG  
 55 GAGGCATTTGGTGTACTGTCGTGTTGGCTGCTGGCATCCAGTGGCAGAGGCCAGGGACACT  
 GCT  
 CAGCATGGTACAGTCACAGGACAGGCCATCATCAAAGAATTATCTGGTCCAAATGTCATAGTTGAGCAT  
 TG  
 AGAGACCCTAGCCTTCACTTAAGTGGGCGATTGCTCCGTTGGTGTGTCGTCAAAACCTTGTCC  
 A  
 60 GGTACTGGGAGTGTCAACTAGAGCCAGGAATTGGGAGCCGTTGGTGTGTCGTCAAAACCTTGTCC  
 TTT  
 CTGTCGGCAAGCTAGTATCCATTAGGTACCTCAGGAACCCAAATGATTTGTCAATAACAGGAATGT  
 GA  
 65 GCACACTGAAGACATTTAAGAAGGCTCATTGTCAGCAGAAATTTCAGTGTACTAGTGGCATTATAGAAA  
 GA  
 GAAGGTGATCACTGAAGGCATGTCACATAATATTCTGAGCCCTGGTGGCGTTATCTAGGGCAAGGATTC  
 CAC  
 CTGTGTTGGAGTTGCGCCCATCCTCACTGTAGCCAGAGCTTCTCTATCAGAGTTAGTATTTGTTGAATA  
 GA

GGATCTTGCTGTTAAAACAGTTGAAAGACCCGTATGGCAGGCCGTATTGACAAGCGAATGATGGGAACA  
 TGA  
 ATCGGTCTTAGGGAAAGCATCTGTCAAAGTGGCCTGGTTAAAACAAGTGCCTCCTCTCAGTGTCACTTGA  
 TT  
 5 GTGTGCTTGAATTCTCGGAAAACGGGTATGAGACCCACGATGAATTGCCACACGATTGATTGGACTCT  
 TC  
 CTTCACCTGCTCTCAGCCAGTGCAGTCCTTCTGATCATGTGATTGACGTGAGAACGTAGTCTGTATAT  
 CA  
 10 AATCTTAAAGAATGTTTGAGTTCTGGACACAGGAACCCAGCACTAGCATACTACAAATCTAATGTCTT  
 AA  
 TGGCATCATAAAAAGAGGCTTAAACACAGACTCCAGTAGCTAAGTGGTTCTGCTAGTGCCTGACTGTTGC  
 AG  
 15 GGGCCCTGTGAGATGCCAGTCCCTGAAAGAAATGAAAAGGCCAGTTACCGTAGGTGGTGTGGAAAACAT  
 GGG  
 CTAGATCATCAGGCAGGACAGAATGCCCTGGCTGTGGTGGAGCACCCAGCTGGCTGAGTTCTGGTTCT  
 ACC  
 ACTGCGTTGTTGTGACCAATTATGAGTTGCTTAACCTTCTTGCTACTATTCCTGTTGCAAAATGGTTC  
 A  
 20 TTGACCCCTGTCTTCCACCTCCCAGGACAATTCAACAGCTTATTTGTAAGAAAAGATCACAGTCCTTAAAAAAT  
 A  
 TAACTGTAAAGTCAGAGGTATGCTTGAAGAGCAGGAACCAGGTAGATGTGGAAATGTCATGTCTTGTTC  
 TAA  
 AGAAAAGGCATTCATAGCTTTGGATATGACGCAACATACCAAAATCCTGACACATAGTGGAGTCGGAA  
 AT  
 25 TGCAACAAACGCCAGTTATAAACCCAGCTAGTTGGGTATGATTGTAAGAAAAAAAGCTGCCATTCTGATT  
 TG  
 GGGAAATTGATTTCTAACTTATATTCTAGTAGCTAGATTATCATATTGTACTATCATCTGGCTTTTT  
 AAGACTTAAAGAAGATCAAGTAAATTTTTCTTCTTCTTACAGACTATAGATCATCAAGGGTGTCTGCTTA  
 C  
 30 AGGTGGATAGTGTATGATCTACAGTGAGGGGACATTATTAAAACATTACATGTGTTTGGGGTGG  
 TA  
 TTTAACGGCAGCACCTCTGATTGCTTGGAGGGCTGGTGTGTTGAAGTTCTGCTCTCCCTCAGTGG  
 CT  
 35 CTAACCTCTGATGCACGTGAGACACATTGCTTGTGAGAAACTAAAGCAAACACTGTCTG  
 GG  
 GACAGGTTTCATTGTCAGATCTTCTGCCACATGAGTGTGTTGGACAATACAGCTGCTTCCAAA  
 TT  
 GCTAAATTGACAGACTTCTAGGTGCTTCCCAATGCCAGACTTCTTCTGTTGAAGATTAAAGTTGTGCT  
 T  
 40 GCTGCCCTCTAGGGTCAGTTGTTAATCTAACCTTAAACGGCTTATTTCTGGTGTGTTGGAAAGTTGA  
 CG  
 GTTGTAAATTGGCTCATTTCTAAATTATTCTGAAGAAGATAATTCTGCCAGTATGTATGTCCACCTCA  
 G  
 45 TTTGCCAGATCCTGCCGTCTCAGAGACACTGAGAACCGGAAGCTGCCGGCAATTCACTATGAAATGATC  
 TTT  
 CTTGTGATTAAGGCAAACGAAGAACTGAATGTTAATAGTGTACTCTGCTGTACCCAGAAAAAAACAAA  
 AT  
 CATGTTATAACACTCTAAACCTCAACCTCAACAGCATTGGTGTGTCTAGCCGTTGTTCTAACCC  
 G  
 50 ATGTTATATAAAAAGAATTCTCATGCTTCCAAAATGTTATGTCAGAATATTAAAGTCAGCATGCCATT  
 C  
 AGGTACTTCAGCTACCTCTTATATAAAATTCTTCTTAAAGATAAAAATGATGATGGAGGAAAAATA  
 A  
 AACATCTTACATCTCAAGAGAGTGTAGTTATGTTGAAAGTCTTCCGTTCAAAATTATTACCTCAATT  
 T  
 55 CAGAACGCATACAGGTAAAGAACTTTATTCTTAAACCATGCTTAAAGATAATTATGTTATCTAATT  
 T  
 GTTGTGTTCTCAGATACTCTGCCAGATCTGGACTAGCTTAAAGGATAATTATGTTGATGCTGAGT  
 GG  
 60 TTATTTTATCTTCTTACTGCTTAACTGTAACCTGAGCCATTGTTCTTATTGCAATTCTTCTTCTT  
 TGTTCTTGTGAGACGGAGCTTGCTCTGTCACCTCGGCTGGAGTGCAGTGGTCAATTGGCTACTGCAGCCT  
 CC  
 ACCTCCCTGGTCAAGCAATACTCCTGCCCTAGCCTCCCCAGTAGTTGGATTACAGGTACCTGCCACCA  
 CG  
 65 GCTAATTCTGTATTTAGTAGAGATGGGGTTTACCATGCTGGCCAGGCTGGTTGAACTCCTGACCTCAA  
 GT  
 GATCCGCTACCTTGGCTCCCATAGTGTGGCTCCCATAGTGTGGATTACAGGCGTAGGCCACCGGCC  
 CGG  
 70 ACAAAAGTTCAATTGTTAGTTATGACTGCTATGTCTGACTCTTATTTAAAGCTACAGTATT  
 G

CTGCATCTTATGTCTTATGATTGAGAATGAAATGAGAATCTATTTAGTAGCTTGAGATTGTGAAAGGAGCTA  
 5 TG  
 ACATCATGATGAGGAGGCTCGTAGATTGAAATTCTCTCCACTTACTATCTGTGACCCCTGGGCAA  
 GT  
 TATTAACCTTTGTGTTTAGTTCTTGTAAAAGTAGAATAATACATATTCCTAGGGCTTCTT  
 A  
 AGATTAATAAGTTAGAAGTGTGCTTAATTCTATTGAAGATAGGCATTCTATAATTCAAATATTCTT  
 C  
 AGTAAGGATGATAAAGAACTGATGAGAAATCTATGTGATAGTAGATCGAGAAAGCAAAAGGAGGAAAGC  
 10 CTG  
 TTTCTTAATAAAATAGATATTGATCTATTCTAGTCTTCTACACTCTATAATAAAAGTGCCTTCTGCT  
 TAGGTGAAAAACCATAACAAATGTGAATTCTGTGAGCCCAGAACAGACATCTGTGAGGTATCTG  
 GA  
 GAGACATCACAGGAAAAACAAACCGATGTTGCTGCTGAAGTCAGAACGATGGTAAAATCAGGACACTGAA  
 15 GAT  
 GCACTATTAAACCGCTGACAGTGCCTAAACCAAAAATTGAAAGATTCTGTGCTGCCAAAGATGTTACAGG  
 CA  
 GTCCACCTGCAAAGCAGCTTAAGGAGATGCCTCTGTTCTAGAATGTTCTGGCAGCGCTGCTCTCACCA  
 GC  
 20 ACACAAAGATACTCAGGATTCATAAAAATGCAGCTGATGACAGTGTGATAAAGTGAATAAAACCTACCC  
 CT  
 GCTTACCTGGACCTGTTAAAAAGAGATCAGCAGTTGAAACTCAGGCAAATAACCTCATCTGTAGAACCAAGG  
 CGG  
 25 ATGTTACTCCTCCCTGGATGGCAGTACCAACCCATAACCTTGAAGTTAGCCCCAAAGAGAACAAACGGAGAC  
 CGC  
 AGCTGACTGCAGATAACAGGCCAAGTGTGGATTGTCAAGAAAAACCTTAAATTATCCGTGGGGCTCTCACCA  
 AT  
 TGCCCGGCAATTCTTGAGTAAAAGTTGATTCCAAGTATCACCTGTCCATTGTTACCTTCAAGACATTAT  
 C  
 30 CAGAAGTTTAATGTCACCAAGAGACTGGACCATAAATACAATCCTGACGTTCTATTTAACTGTCGAAACAAG  
 TC  
 CTTGCTTAGAGTCGACGTACCGGATGCCGCCAGCGTTGCTGGAAAAGATGTGCCTCCCCCTCTAGTTCT  
 TGT  
 35 AAACCCAAGCCAAGTCTGCTTCCCGCGCAGTCAAATCCCTGCCATCTGCCAAGGGGAAGCAGGCCCTC  
 CTG  
 GGCCAGGCAAGGCCCTCTGACTTCAGGGATAGACTCTAGCACTTAGCCCCAAGTAACCTGAAGTCCCACAG  
 ACC  
 ACAGCAGAATGTGGGGTCCAAGGGGCCACCAGGCCAACAGCAATCTGAGATGTTCTAAACCAAGTGT  
 TCC  
 40 CCTGCACCGATAAGACAAAAAGACCCGAGACAAAATTGAAACCTCTCCAGTAGCTCTCTCAGCCCACCC  
 CG  
 GCAGCAGTAACATCAATGGTCCATCGACTACCCGCCAACAGCACAGCCCCGGCACCTCCGGGAAGAGA  
 CTA  
 TTCTGTAATGGAGTGCAGCAATACTGCAGCAGAAATTGGTGAGCCCCCTCCAAAAGACTGAAGTCCAGC  
 45 GTG  
 GTTGCCCTTGACGTTGACCAGCCGGGCAATTACAGAAGAGGCTATGACCTCCCAACTACCATATGGTCA  
 GAG  
 GCATCACATCACTGTTACCGCAGGACTGTGTATCCGTGCGAGGCCTGCCAAACCAAGGTTCTGAG  
 CTC  
 50 CAGCGAGGTGATTCTCAAATGTGCTGACTGTTAGAACCCCTATGGTGGCTCCGGGCCACTTACACTTGT  
 GTG  
 CCTGCTGGTAGTCCAGCATCCAGCTGACGTTAGAAGGTATTGATGAGGGGCGTGTGTTAAATGGCTGCC  
 TAC  
 AGTGATTAATAGCTAATCCAGGATTCAGTGGAGATGGTACCAACTCCAAAGGGTGGGGGTAGGCAGCCAG  
 AAG  
 55 TTCTGGGGTCACAGAGAGAACATCTTAGATACGGCAGTGGTTGTGGCTCCAAAGGCTTACTTAACCT  
 GT  
 GGGTTAACTCTAACCCGTGTATTCTTTGATTGTTAGTCITACTTTATTAGAGAAAGGGTCTT  
 GCTCCGTCTAGATTGGAGTGCAGCGGTGTAATCATAGCTTACTGTAGTCATGAAATTCTGAGTTCAAGAGA  
 TC  
 60 CTTCTGCCCTCAGCTCCAGGTAGCTGAGACTATATGTGCTGCTACCATGCACAGCTGATTAAATT  
 G  
 TAGAGATGGAGTTGCCAGGCTGGTCTGAACTCCTGGCCTGAGGTGATCTCTGCGTGTGACCTCCAAAGTA  
 TCT  
 65 TAGACTACAGATGCACTCCACCACCGCTTG

SEQ. ID. No. 10

ZABC1 Open reading frame

ATGCAATCGAAAGTGACAGGAAACATGCCAACTCAATCCCTTTAATGTACATGGATGGGCCAGAACGTGATTG  
GCA

GCTCTTGGCAGTCGATGGAGATGGAGGATGCCCTGTCAATGAAAGGGACCGCTGGTTCCATTCCGAGC  
 5 TAC  
 ACAAGAAAAAAATGTCATCCAAATCGAGGGTATATGCCCTGGATTGCATGTTCTGCAGCCAGACCTTCACAC  
 AT  
 TCAGAAGACCTTAATAAACATGCTTAATGCAACACCCGGCTACCCCTCTGAACCAGCAGTTCTCGGGTTGA  
 AG  
 CAGACTATCTCAGTCGCTTGATAAAAGTCAGTGCAGAACAGAACCTCCAAGGAAAAGAATTGCAAGGAAA  
 TGA  
 10 ATTAGCTGTGAGGTATGTGGCAGACATTAGAGTCGTTTGATGTTGAGATCCACATGAGAACACACAAAAG  
 AT  
 TCTTCACTTACGGGTGTAACATGTGCGGAAGAAGMTTSRRSSAGCCTGGTTCTTAAAAATCACATGCGGAC  
 AC  
 15 ATAATGGCAAATCGGGGCCAGAACGAAACTGCAGCAAGGCTGGAGAGTAGTCCAGCAACGATCAACGAGG  
 TCGT  
 CCAGGTGCACGGCCGAGAGCCTCTCCTTACAAAATCTGCATGGTTGTGGCTTCTTATTTCAAATA  
 AA  
 GAAAGTCTAATTGAGCACCGCAAGGTGCACACCAAAAAACTGCTTCGGTACCCAGCAGCGCGAGACAGACT  
 CTC  
 20 CACAAGGAGGAATGCCGTCTCGAGGGAGGACTTCCTGCAGTTGTTCAACTTGAGACCAAAATCTCACCCCTGA  
 AAC  
 GGGGAAGAAGCCTGTCAGATGCATCCCTCAGCTCGATCCGTTACCCACCTCCAGGCTGGCAGCTGGTACCC  
 AAA  
 25 GGAAAAGTTGCCATTGCCAAGAAGTGAAGGAATCGGGCAAGAAGGGAGCACCGACAACGACGATTGAGT  
 TCCG  
 AGAAGGAGCTGGAGAAACAAATAAGGGAGTTGTGCAGGCCTCTGCAGAGAGAAAGAGAAGTGCACAAACT  
 CCCA  
 30 CGCGGAAGCGCCCTCCGTGGACCGGATCCAAGTTACCCAGTAGCAAGGAGAACCCACTCACTGCTCCGA  
 GTGC  
 GGCAAAGCTTCAAGAACCTACCAACAGCTGGTCTTGCACCCAGGTCCACAGAGAACGGACGGAGGGCCGGC  
 GCGG  
 AGTCGCCACCATGTCGTGGACGGAGGGAGCCGGAGCTGTTCTCCTGACCTCGCCGCCCTCTGGATGA  
 AAA  
 35 TGGAGCCGTGGATCGAGGGGAAGGTGGTCTGAAGACGGATCTGAGGATGGCTTCCGAAGGAATCCATCT  
 GGAT  
 AAAATGATGATGGAGGAAAAATAAACATCTTACATTTCAAGAGAGTGTAGTTATTGAAAGTTTCCG  
 TT  
 CAAATTATTACCTCAATATTCACTCAGAACGCATAACAGGTAAAAACCATACAAATGTGAATTITGTGAATAT  
 GC  
 40 TGCAGCCCAGAACGACATCTCTGAGGTACTTGGAGAGACATCACAGGAAAAACAAACCGATGTTGCTGCT  
 GAA  
 GTCAAGAACGATGGTAAAATCAGGACACTGAAGATGCACTATTACCGCTGACAGTGCAGAACCAAAATT  
 TGA  
 AAAGATTTTGATGGTCCAAGATGTTACAGGCAGTCCACCTGCAAGCAGCTTAAGGAGATGCCCTCTGTT  
 TT  
 45 TCAGAATGTTCTGGCAGCGCTGCTCTCACAGCACACAAAGATACTCAGGATTTCCATAAAATGAGCTG  
 AT  
 GACAGTGTGATAAAAGTGAATAAAACCTACCCCTGCTTACCTGGACCTGTTAAAAAGAGATCAGCAGTTGA  
 AA  
 50 CTCAGGCAAATAACCTCATCTGAGAACCAAGGCGGATGTTACTCCCTCCGGATGGCAGTACCAACCCATAAC  
 CT  
 TGAAGTTAGCCCCAAAGAGAACGAAACGGAGACCGCAGCTGACTGCAGATAACAGGCCAAGTGTGGATTGTCAC  
 GAA  
 55 AAACCTTTAAATTATCCGTGGGGCTTCAACATTGCCCCGCAATTCTTGTAGTAAAAGTTGATTCCAAG  
 TA  
 TCACCTGTCCATTGTTACCTCAAGACATTATCCAGAAGTTTAATGATGCACCAAGAGACTGGAGCATAAA  
 TA  
 CAATCCTGACGTTCATAAAAGTGTGAAACAAAGTCCCTGCTTAAAGTGCACGTACCCGATGCCCGCAGCG  
 TTG  
 60 CTGGGAAAAGATGTGCCCTCCCTCTAGTTCTGTAACCCAAAGCCAAAGTCTGCTTCCGGCCAGTCCAA  
 AT  
 CCCTGCCATCTGCGAAGGGGAAGCAGAGCCCTCTGGCCAGGAAGGCCCTCTGACTTCAGGGATAGACTC  
 TAG  
 CACTTAGCCCCAAAGTAACCTGAAGTCCCACAGACCAAGCAGAACATGTGGGGTCAAGGGGCCACCG  
 CAA  
 65 CAGCAATCTGAGATGTTCTAAACCCAGTGTTCCTGCACCCGATAAGACAAAAGACCCGAGACAAAATT  
 GA  
 AACCTCTCCAGTAGCTCTCAGCCCCACCCCTGGCAGCAGTAACATCAATGGTCCATCGACTACCCGCC  
 AA  
 70 GAACGACAGCCCCTGGCACCTCCGGAAAGAGACTATTCTGTAATCGGAGTGCAGCAATACTGCAGCAGAA  
 TTT

5 GGTGAGCCCCCTCCAAAAGACTGAAGTCCAGCGTGGTGCCCTGACGTTGACCAGCCCCGGGCCAATTACA  
 GAA  
 GAGGCTATGACCTTCCCAGTACCATATGGTCAGAGGCATCACATCACTGTTACCGCAGGACTGTGTATCC  
 GTC  
 GCAGGGCTGCCCTCCAAACCAAGGTTCTGAGCTCCAGCGAGGTGATTCTCAAATGTGCTGACTGTTAG  
 AAG  
 CCCATGGTGGCTCCGGGCCACTTACACTTGTGCTGCTGGTAGTCCAGCATCCAGCTGACGTTAGAAG  
 GTC  
 TTGGTGGATGTCAGTGCTTACTCCCCATGAAATTAAATTACTTCATCCTTGAGAAGCGAATGGTAAAGCT  
 10 AC  
 TGAATAAGCTGTGATTGACTGTACATAAACATATGAGGAATCTGCAAGGAACACTACAGTTGTGAA  
 SEQ. ID. No. 11

ZABC1 Protein

15 MQSKVTGNMPTQSLLMYMDGPEVIGSSLGSPMEMEDALSMKGTAVVPFRATQEKNVIQIEGYMPLDCMFCSQTFT  
 H  
 SEDLNKHVLMQHRPLCEPAVLVEAELSPLDKSQVRTEPPKEKNCKENEFSCEVCQQTFRVAFDVEIHMRTHKD  
 SFTYGCNCGRXXXXPWFLKNMRTHNGKSGARSKLQQGLESSPATINEVVQVHAAEISSPYKICMVCGLFPNK  
 ESLIEHRKVETIEKTAFGTSSAQTDSPQGGMPSSREDFLQLFNLRPKSHPETGKKPVRCIPQLDPFTTFQAWQLATK  
 20 GKVAJCQEVEKGQEGSTDNDDSSKELEGETNKSCAGLSQEKEKCKHSHGEAPSVDADPKLPSSKEKPTHSEC  
 GKAFTYHQLVLRSLRVRKKDRRAAESPTMSVDGRQPGTCSPDLAFLDENGAVDRGEGGSEDGSEDGLPEGIHLD  
 KNDDGGKIKHLTSSRECSYCGKFRSNYYLNIRLRTHTGEPKYCEFCEYAAAKTSLRYHLERHHKEKQTDVAE  
 VKNDGKNQDTEALLTADSAQTKNLKRFFGAKDVTGSPPAKQLKEMPSVFQNVLGSAVLSPAHDKTQDFHKNAAD  
 D  
 25 DSADKVKNKPTPAYLDLLKKRSAVETQANNLICRTKADVTPPPDSSTHNLEVSPKEKQTEAACDCRYRPSVDCH  
 KPLNLSVGALHNCPAISLSSKLIPSITCPCTFKTFYPEVLMMBQRLEHKYNPDVBKNCRNKSLLRSRRTCPAL  
 LGKDVPPLSSFCPKPKSAFPAQSKSLSPSAKGKQSPPGPGKAPLTSGIDSSTLAPSNLKSHRPQONVGVQGAATRQ  
 QQSEMFPKTSVSPAPDKTRPETYKLPVAPSQPTLGSSNINGSDYPAKNDSPWAPPGRDYFCNRASNTAAEF  
 30 GEPLPKRLKSSVALDQPGANYRRGYDLPKYHMVRGITSLLPQDCVYPSQALPPKPRFLSSVEDSPNVLTQK  
 PYGGSGPLYTCVAGSPASSSTLEGGLGCQCCLPMKLNFTSSFEKRMVKATEISCDCTVRKTVESARNTTVV

## SEQ. ID. NO. 12

1b1

35 GGAAACAGCTATGACCATGATTACGCCAAGCTGAAATTAAACCTCACTAAAGGAACAAAAGCTGGAGCTCC  
 ACC  
 GCGGTGGCGCCGCTCTAGAACTAGTGGATCCCCGGGCTGCAGGAATTGGCACGAGGCTCCACCGACAGC  
 CAGG  
 CACTGGCAGCACCACTGGAGACCCAGGACCCCTGTGCAGGAGCAGCTCCGGGTGACACGGGGACTGAAG  
 ATAC  
 40 TCCCACAGGGGCTCAGCAGGACAATGGTAACCAAATGAGTGTCCCCAAAGAGTTGAAGACCAAGAGAATG  
 AAC  
 CAGAAGCAGAGACTTACCAAGGACAACCGTCTGCTCTGAACGGGTTCCAGTGGGGTGTGACCCACACAGT  
 TCA  
 45 GCACCTAGAGGAAGTCGACTTGGAAATAAGTGTCAAGACGGATAATGTGCCACTTCTCCCCGAGACAACG  
 GAG  
 ATAAGTGTGCTGCGGATGCCAACCGAAAGAAATCTTGGAAAGAGGCCAAACCCGAGGCACCAAGCTGCTAAAT  
 CTC  
 GTTTTTCTTGATGCTCTCGGCCTGTACCAAGGACGTACGGAGACCAAGCCGAGATTCACTCCCTGGATCA  
 GT  
 50 GAACCTTGATGTCAGCTCCAATAAGCTCCAGCGAACAAAGACCCAAAGTGAGAGCTGGACACTTCCGGTGGCA  
 GCT  
 GGACCGGGGAGGACACAGATAAAACCCAGGGCACGCCCGGCCAAGACAAGGTCTCTGCCAGG  
 GATC  
 CCACGTTCTCCCACCTGAGACAGGGGGAGCAGGAGGAGAAGCTCCCTCAAGCCAAAGGACTCCAGCTTTT  
 TGA  
 55 CAAATTCTCAAGCTGGACAAGGGACAGGAAAGGTGCCAGGTGACAGCCAAAGGAAGGCCAAGAGGGCAGA  
 GCAT  
 CAAGACAAGGTGGATGAGGTTCTGGCTTATCAGGGCAGTCCGATGATGTCCTGAGGGAAAGGACATAGTT  
 ACG  
 60 GCAAGGAAAAAGAAGGACAAGAACTTGGAACTGCCGATTGCTCTGTCCTGGGACCCAGAAGGACTGGAGA  
 CTGC  
 AAAGGACGATTCAGGCAGCAGCTATAGCAGAGAATAATAATTCCATCATGAGTTCTTAAACTCTGGTTT  
 CA  
 CCTAACAAAGCTGAAACAAAAAGGACCCAGAAGACACGGGTGCTGAAAGTCACCCACCACTCAGCTGACC  
 TTA  
 AGTCAGACAAAGCCAACCTTACATCCCAGGAGACCCAGAAGACACGGGTGCTGAAAGTCACCCACCACTCAGCTGACC  
 GGG  
 GCACACACAGTCGTGACAACCCCTGAACCTGCGAAGGAAGGCACCAAGGAGAAATCAGGACCCACCTCTG  
 CCT

CTGGGCAAACGTGGAAAAGTCAGTAAAGAGGACTCAGCCCCACAGGTGCCAGGAGAATGTGGTGT  
 GTG  
 AGTCACCACTAGAGATTATAAGTCCAAGGAAGTAGAACAGCCTTACAAACAGTGGACCTAACGAAGGAGA  
 TGC  
 5 TGCAACCTGAACCCACAGAAGCGAAACTCAAAGAGAAGAAAGCAAACCAAGAACCTCTGTATGGCTTTCTC  
 AGA  
 CAAATGTCAGTGAAAGGGATGGAGGGATCACCCACTCAGAAGAAATAATGGGAAAGACTCCAGCTGCCAAA  
 CAT  
 10 CAGACTCCACAGAAAAGACTATCACACCGCCAGAGCCTGAACCAACAGGAGCACCACAGAAGGGTAAAGAGG  
 GCTC  
 CTCGAAGGACAAGAAGTCAGCAGCCGAGATGAACAAGCAGAAGAGCAACAAGCAGGAAGCCAAGAACCAAGC  
 CCAG  
 TGCACAGAGCAGGCCACGGTGGACACGAACACTCACTGCAAGATGGGACAAGCTCCAAAAGAGACCTGAGAAG  
 CGGC  
 15 AGCAGTCCCTGGGGCTCTTAAAGGCTGGACCAAGCGATGTTGATGCTCAAGTCAAACAGACCC  
 AGT  
 ATCCATCGGACCAGTTGGAAACCCAAGTAAACAAATCAGCACGGTCCCACCAAGGTTCTCTGCCACCAAGA  
 TGT  
 20 GTTCTCTTACTCCATCTCCCTCCCCAAACACGGCTCCATGTATATATTCTCTGATGGCCAGCAAATGAAATTCTG  
 C  
 CTAGAAATTAAAGCCCAGCTGTTGATATTGAGGTGATTATTACGTCTGGTCCAGTCTTCTGGCAAAT  
 AA  
 CAGTAAGATGGTTAGCAGGTACCTAGTTGGTCAGAAGAGTCGATGATCACCAAGCAGGAAGGGAGGG  
 AATA  
 25 GAGGAATCTGTTGGGTTAAGTGTGAAATGGCAGTGGTGGCCGGCGTGGTGGCTCTGCCCTGTAATCTCA  
 GCA  
 CTTTGGGAGGCCGAGGCAGGTGGATCACCTGAGGTCAAGACTAGCCTGGCAACATCATGAAACC  
 CCG  
 30 TCTCTACTAAAAATACAAAATTAGCCAGGCATGGTGGCACACACCTGTAGTCCCAGCTACTCGGAGCCAA  
 CGC  
 ACGAGAACCGCTTGTACCCAGGAGGTGGAGGTTGCAGTGAGCCGAAGTTGCACCATTCGACTCCACCCCTGGC  
 GAC  
 AGAGCAAGATTCTATCAAAAAAAAGGCAGTGGCAAGTAAGTTATAGAAGAGAAATGCTGCTAGAAGGAATT  
 AAG  
 35 CGTTGTAGTAAACCGGTGCTCATCCTCTAACGTTGAAGAAGGGAGACGAAATCCATTGTTAAATTACATC  
 TC  
 AAGGAGGGAGAACCCGGCTGTGTTGGGTGGTGCCTAGAACGGAATGTGTTGGGTATAGAAAAAA  
 GGAA  
 40 TGAATAAGCGTTGTTTCAAATAGGGCTCTGTAAGTTATTGATGAGAGGGAAAAGATTGACTGGGGAGGGC  
 TTA  
 AAATGATTGGAAAACAATTGCTTTGAGGCTCAGTGACAACGGCAAAGATTACAACCTAAAAAA  
 AA  
 AAACTCGAGACTAGTTCTCTCTCTCGTGCCGAATTGATATCAAGCTTATCGATACCGTCACCTCGAGG  
 GG  
 45 GGGCCCGGTACCAATTGCCCTATA

## SEQ. ID. NO. 13

Genomic Sequence from BAC clone 97

Filtered query sequence:

&gt;query\_seq

5        TGTGATATTGATTCATGCCCTTGCACCTGCCAACATCACACGCTTG  
       CCATCCAGTCCACTCGATTTGGCAGTGCAGATGAAAAACTGGGAACCAT  
       TTGTGTTGAGTCCAGCAAGATGCCAGGACCTGCATGTTCAGAACGAAGT  
       TCTTCATCATCCAATTCTCCCTGTATATGGGCTTACACNACTGCCGT  
       AAGTCGTGTNAAGTCACCACTCAGGTACATAATGGAATAATTCTGCAAAG  
 10      GCAGGAGNCACTTCTCCAGTGCTCAGACCATGAAAGTTTCTGATGT  
       CTTTGGAACTTGTCTGCAAATAGCTGAAGGAGACATGGCCTAAAGGCT  
       CGCCATCTGCGGTGATATTGNAACATGGTAGGGCTGACCGTGGCTGTGGC  
       CATGACTTTAGANTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  
       N  
 15      NNN  
       NNN  
       NNNNNNNNNNNNNNCCAATGCGGGACAGAGAATCNAAGAAACTGTATT  
       GGGAAAGGGTCTGAGTTATGCCAAAGTTCCCAGATTGGTTCCATTG  
       AACACGTAGCTCTGTGAGATACCATCAGGTGTTATGTGAAGAAATGTCTGT  
 20      GTAGTCAAATATGTTGAGTGAGTGAGCTGAGCTGAGCAAGACTTTACT  
       GCAAGACTTCCCATTTCTGTCCCTTTATGCTAATGGTAACACAAAC  
       TCCAAAAGTGGGTGTACAGCATGAGGCATTAACAAAAATTATTGGACC  
       CCACACACNN  
       NN  
 25      NNN  
       NNN  
       NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTCTC

## SEQ ID NO 14

gb|M19533|RATCYCA Rat cyclophilin mRNA, complete cds.

Length = 743

Minus Strand HSPs:

35      Score = 418 (115.5 bits), Expect = 1.5e-58, Sum P(5) = 1.5e-58

Identities = 96/112 (85%), Positives = 96/112 (85%), Strand = Minus / Plus

S = rat CYCLOPHILLIN; q = SEQ ID NO 13.

Query: 372 TNCAATATCACCGCAGATGGCGAGCCTTCTGGCCATGTCTCTCGAGCTATTGAGAC 313

40      Sbjct: 64    |||TCGACATCACGGCTGATGGCGAGCCCTGGGCGCTGCTGAGCTGTTGAGAC 123

Query: 312 AAAGTTCAAAGACATCAGAAAACCTTCTGGCTGAGCACTGGAGAGAAAG 261

45      Sbjct: 124 AAAGTTCAAAGACAGCAGAAAACCTTCTGGCTGAGCACTGGGGAGAAAG 175

Score = 236 (65.2 bits), Expect = 1.5e-58, Sum P(5) = 1.5e-58  
 Identities = 52/58 (89%), Positives = 52/58 (89%), Strand = Minus / Plus

Query: 117 TGCTGGACTCAACACAAATGGTCCCAGTTTCTGCACTGCCAAAATCGAGTGG 60  
Sbjct: 348 TGCTGGACCAACACAAATGGTCCCAGTTTATCTGCACTGCCAAGACTGAGTGG 405

5 Score = 177 (48.9 bits), Expect = 1.5e-58, Sum P(5) = 1.5e-58  
Identities = 41/48 (85%), Positives = 41/48 (85%), Strand = Minus / Plus

Query: 60 GACTGGATGGCAAGCGTGTGATGTTGGCAAGGTGCAAGAGGGCATGA 13  
Sbjct: 404 GGCTGGATGGCAAGCATGTGGCTTGGAAGGTGAAAGAAGGCATGA 451

Score = 154 (42.6 bits), Expect = 1.5e-58, Sum P(5) = 1.5e-58  
Identities = 34/38 (89%), Positives = 34/38 (89%), Strand = Minus / Plus

15 Query: 153 AGAACCTTCGTTCTGAAACATGCAGGTCTGGCATCTTG 116  
Sbjct: 299 AGAACCTTCATCCTGAAGCATAACAGGTCTGGCATCTTG 336

20 Score = 86 (23.8 bits), Expect = 1.5e-58, Sum P(5) = 1.5e-58  
Identities = 22/28 (78%), Positives = 22/28 (78%), Strand = Minus / Plus

Query: 256 TCCTGCCTTGCAGAATTATTCCATTAT 229  
Sbjct: 193 TCCTCCCTCACAGAATTATTCCAGGAT 220

25

**WHAT IS CLAIMED IS:**

1  
2     1. An isolated nucleic acid molecule comprising a polynucleotide sequence  
3       having a subsequence which specifically hybridizes under stringent conditions to  
4       a sequence selected from the group consisting of SEQ. ID. No. 2, SEQ. ID. No.  
5       3, SEQ. ID. No. 4, SEQ. ID. No. 5, SEQ. ID. No. 6, SEQ. ID. No. 7, SEQ.  
6       ID. No. 8, SEQ. ID. No. 9, SEQ. ID. No. 10, SEQ. ID. No. 12, AND SEQ.  
7       ID. No. 13.

1              1. The isolated nucleic acid of claim 1, wherein the  
2        subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3              2.

1              2. The isolated nucleic acid of claim 2, wherein the  
2        subsequence is SEQ. ID. No. 2.

1              3. The isolated nucleic acid of claim 1, wherein the  
2        subsequence specifically hybridizes to SEQ. ID. No. 3.

1              4. The isolated nucleic acid of claim 4, wherein the  
2        polynucleotide is SEQ. ID. No. 3.

1              5. The isolated nucleic acid of claim 1, wherein the  
2        subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3              4.

1              6. The isolated nucleic acid of claim 6, wherein the  
2        subsequence is SEQ. ID. No. 4.

1              7. The isolated nucleic acid of claim 1, wherein the  
2        subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3              5.

1           8.     The isolated nucleic acid of claim 8, wherein the  
2     subsequence is SEQ. ID. No. 5.

1           9.     The isolated nucleic acid of claim 1, wherein the  
2     subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3     6.

1           10.    The isolated nucleic acid of claim 10, wherein the  
2     subsequence is SEQ. ID. No. 6.

1           11.    The isolated nucleic acid of claim 1, wherein the  
2     subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3     7.

1           12.    The isolated nucleic acid of claim 12, wherein the  
2     subsequence is SEQ. ID. No. 7.

1           13.    The isolated nucleic acid of claim 1, wherein the  
2     subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3     8.

1           14.    The isolated nucleic acid of claim 14, 16, 18, 20, wherein  
2     the subsequence is SEQ. ID. No. 8.

1           15.    The isolated nucleic acid of claim 1, wherein the  
2     subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3     9.

1           16.    The isolated nucleic acid of claim 16, wherein the  
2     subsequence is SEQ. ID. No. 9.

1           17. The isolated nucleic acid of claim 1, wherein the  
2 subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3 10.

1           18. The isolated nucleic acid of claim 18, wherein the  
2 subsequence is SEQ. ID. No. 10.

1           19. The isolated nucleic acid of claim 1, wherein the  
2 subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3 12.

1           20. The isolated nucleic acid of claim 20, wherein the  
2 subsequence is SEQ. ID. No. 12.

1           21. The isolated nucleic acid of claim 1, wherein the  
2 subsequence specifically hybridizes under stringent conditions to SEQ. ID. No.  
3 13.

1           22. The isolated nucleic acid of claim 22, wherein the  
2 subsequence is SEQ. ID. No. 12.

1           23. The isolated nucleic acid of claim 1, further comprising a  
2 promoter sequence operably linked to the polynucleotide sequence.

1           24. The isolated nucleic acid of claim 1, which nucleic acid is a  
2 cDNA molecule.

1           25. A method of screening for neoplastic cells in a sample, the  
2 method comprising:

3                 contacting a nucleic acid sample from a human patient with a  
4 probe which hybridizes selectively to a target polynucleotide sequence comprising  
5 a sequence selected from the group consisting of SEQ. ID. No. 1, SEQ. ID. No.  
6 2, SEQ. ID. No. 3, SEQ. ID. No. 4, SEQ. ID. No. 5, SEQ. ID. No. 6, SEQ.  
7 ID. No. 7, SEQ. ID. No. 8, SEQ. ID. No. 9, SEQ. ID. No. 10, SEQ. ID. No.  
8 11, SEQ. ID. No. 12, and, SEQ. ID. No. 13 wherein the probe is contacted with  
9 the sample under conditions in which the probe hybridizes selectively with the  
10 target polynucleotide sequence to form a stable hybridization complex; and  
11 detecting the formation of a hybridization complex.

1           26. The method of claim 26, wherein the nucleic acid sample is  
2 from a patient with breast cancer.

1           27. The method of claim 26, wherein the nucleic acid sample is  
2 a metaphase spread or a interphase nucleus.

1           28. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 1.

1           29. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 2.

1           30. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 3.

1           31. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 4.

1           32. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 5.

1           33. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 6.

1           34. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 7.

1           35. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 8.

1           36. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 9.

1           37. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 10.

1           38. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 12.

1           39. The method of claim 26, wherein the probe comprises a  
2 polynucleotide sequence as set forth in SEQ. ID. No. 13.

1           40. The method of claim 26, wherein the probe is used to  
2 identify the presence of a mutation in the target polynucleotide sequence.

1           41. A method for detecting a neoplastic cell in a biological  
2 sample, the method comprising:

3                 contacting the sample with an antibody that specifically binds a  
4 polypeptide antigen encoded by a polynucleotide sequence comprising a sequence  
5 selected from the group consisting of SEQ. ID. No. 1, SEQ. ID. No. 2, SEQ.  
6 ID. No. 3, SEQ. ID. No. 4, SEQ. ID. No. 5, SEQ. ID. No. 6, SEQ. ID. No. 7,  
7 SEQ. ID. No. 8, SEQ. ID. No. 9, SEQ. ID. No. 10, SEQ. ID. No. 12, and  
8 SEQ. ID. No. 13; and

9                 detecting the formation of an antigen-antibody complex.

1           42. The method of claim 41, wherein the sample is from breast  
2 tissue.

1           43. A method of inhibiting the pathological proliferation of  
2 cancer cells, the method comprising inhibiting the activity of a gene product of  
3 an endogenous gene having a subsequence which hybridizes under stringent  
4 conditions to a sequence selected from the group consisting of SEQ. ID. 1, SEQ.  
5 ID. No. 2, SEQ. ID. No. 3, SEQ. ID. No. 4, SEQ. ID. No. 5, SEQ. ID. No. 6,  
6 SEQ. ID. No. 7, SEQ. ID. No. 8, SEQ. ID. NO. 9, SEQ. ID. NO. 10, SEQ.  
7 ID. No. 12, and SEQ. ID. No. 13.

1           44. A method of detecting a cancer, said method comprising  
2 detecting the overexpression of a protein encoded in a 20q13 amplicon.

1           45. The method of claim 44, wherein said protein encoded in a  
2 20q13 amplicon is ZABC1.

1           46. The method of claim 45, wherein said protein encoded in a  
2 20q13 amplicon is 1b1.

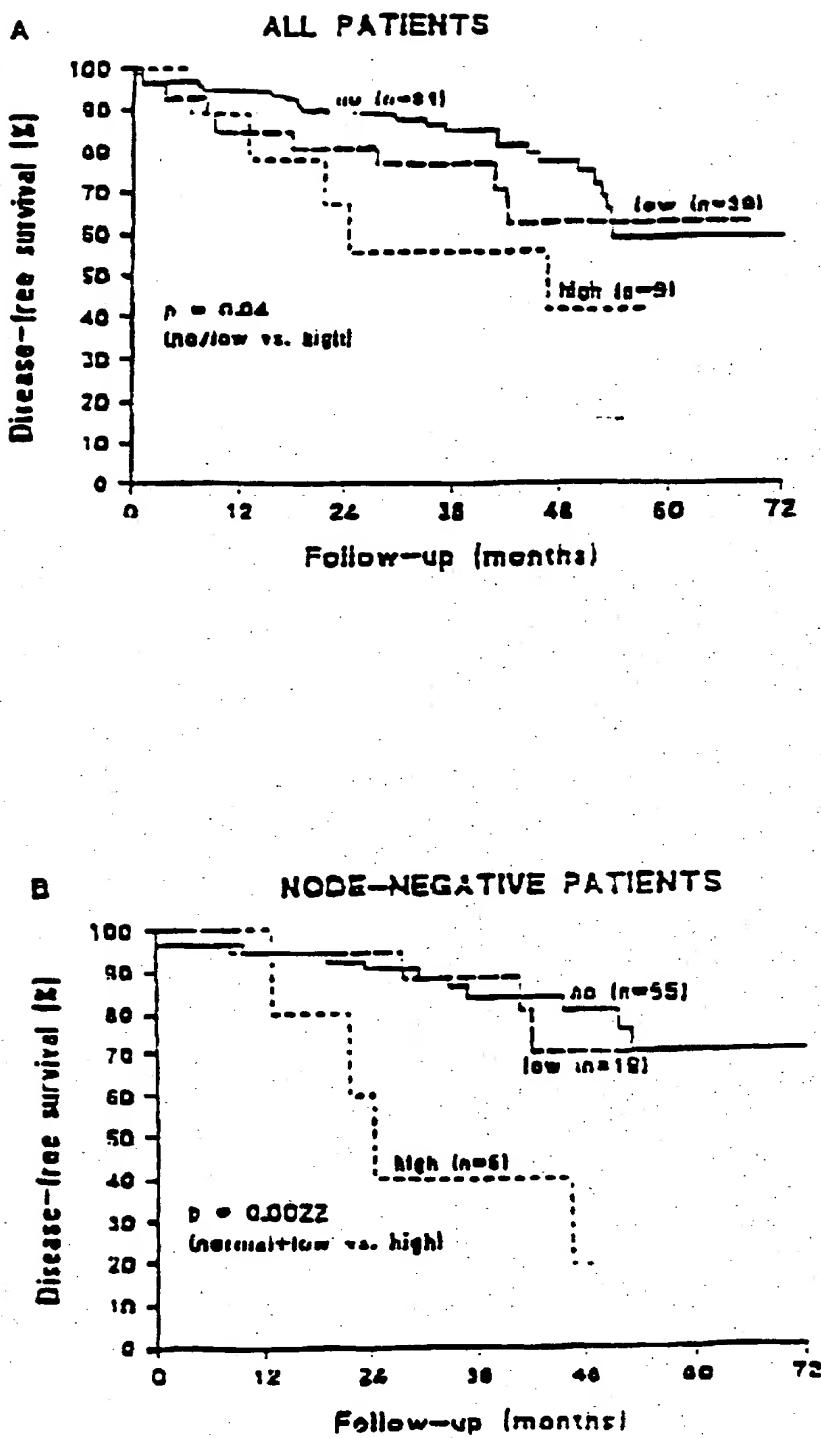


FIGURE 1

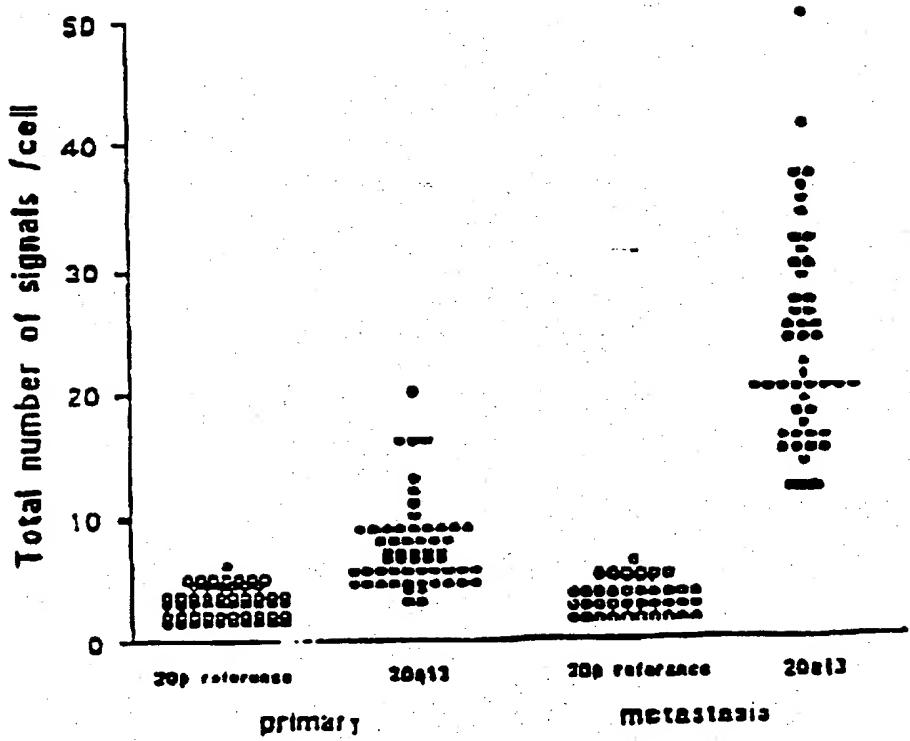
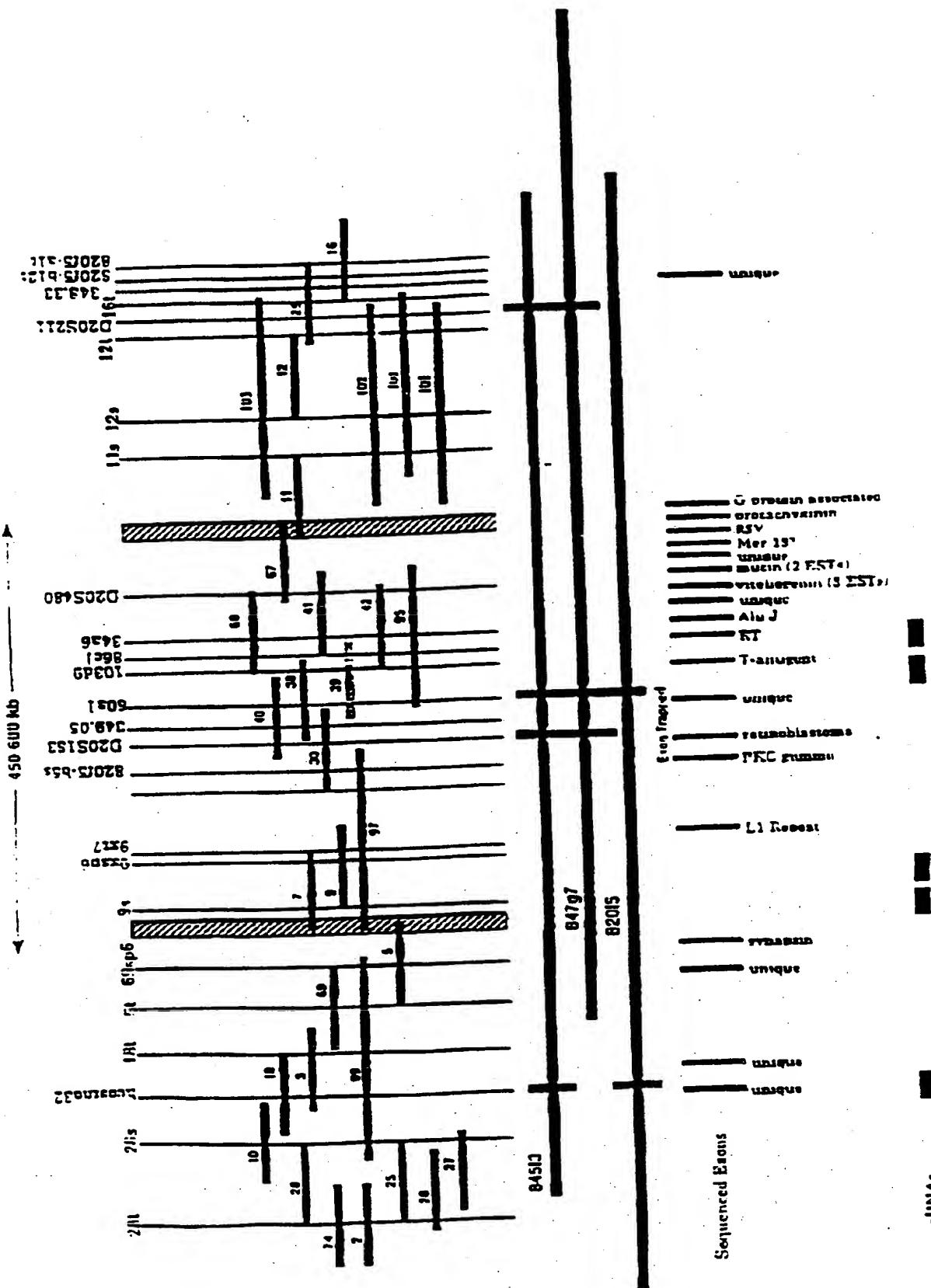


FIGURE 2



**FIGURE 3**

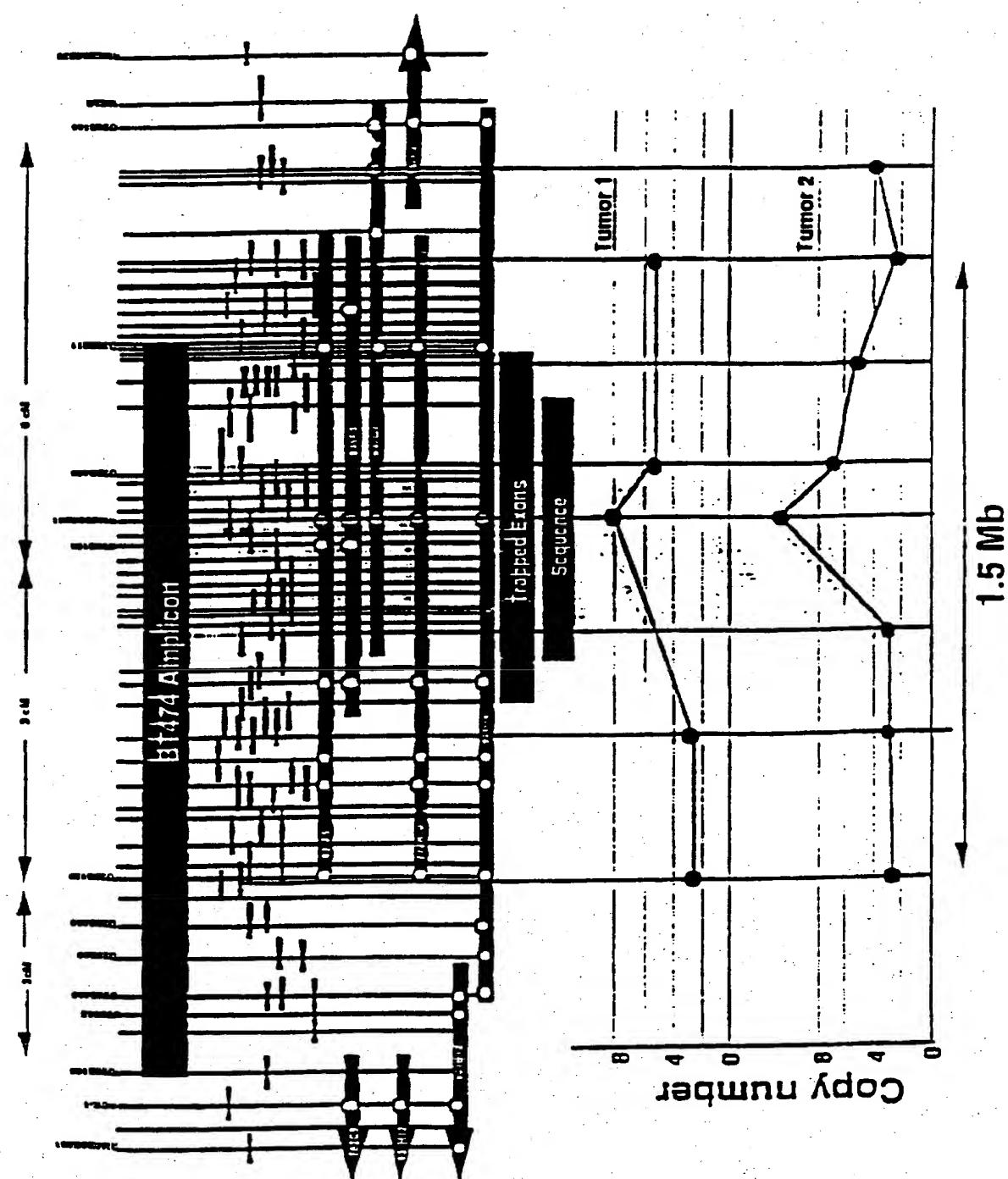
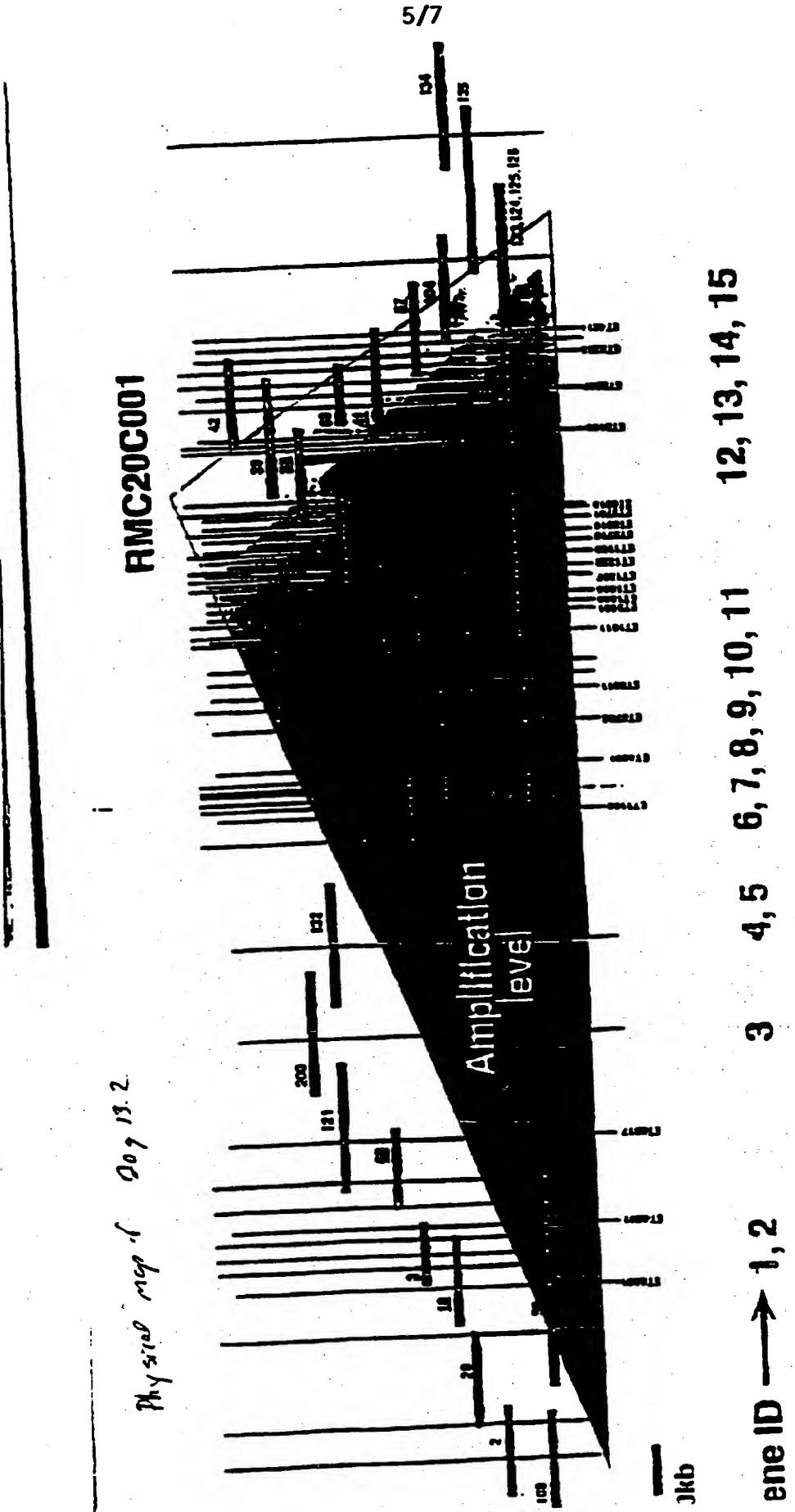


FIGURE 4



6/7

Genomic Sequence from BAC cache 37  
Filtered query by source:

gb|M19533|RATCYCA Rat cyclophilin mRNA. complete cds.  
Length = 743

## Minus Strand ISPs:

Score = 419 (115.5 bits). Expect = 1.5e-58. Sum P(S) = 1.5e-58  
Identities = 96/112 (85%). Positives = 96/112 (85%). Strand = Minus /  
Plus

Query: 372 TNCATAATCACCGCAGATGGCGAGCCCTTAAGGCCATGTCCTCTTCCACGCTATTGCGAGAC  
373

Subject: 64 TCGACATCACGGCTGATGGCGAGCCCTTGGCTGCCTCTGTTGGACTGTTTCAGAC  
123

Query: 312 AAAAGTTCCAAAGACGTCAGAATACTTCATGGCTTGAGCACTGGAGAGAAAG 261

Subject: 124 AAACCTCCAAAAGACATCAGAAACTTCGTGCTTGAGCACTGGGGAGAAG 175

Score = 236 (65.2 bits). Expect = 1.5e-56. Sum P(S) = 1.5e-53  
Identities = 52/58 (89%). Positives = 52/58 (89%). Strand = Minus / Plus

Query: 117 TGCTGGACTCAACACAAATGGTTCCCAAGTTTCACTGCACTGCCAAATCGAGCTGG 60

Subject: 348 TGCTGGACCAAAACACAATGGTCCCAGTTTTTATCTGCACTGCCAAGACTGCTG  
405

Score = 177 (42.9 bits). Expect = 1.3e-53. Sum P(i) = 1.3e-53  
Identities = 41/48 (85%). Positives = 41/48 (85%). Strand = min

Score = 154 (42.6 bits). Expect = 1.5e-58. Sum P(S) = 1.5e-58  
Positives = 34/38 (89%). Negatives = 34/38 (89%). Strand = Minus / Plus

Query: 153 AGAACCTTGGCTCTGAAACATGGAGGTCTGGCAATTG 116

Subject: 299 AGGACTTCATCTTGCAAGCATACAGGTCTGGCACTCTTS 315

Score = 85 (23.2 bits), Expect = 1.04e-58, Sum Z(1) = 1.03e-58  
Identities = 22/23 (78%), Positives = 22/22 (100%), Strand = Minus / Plus

Query: 282 TCCCTGCGTTTGCAGATATTCGAA 229  
Sbjct: 1-97 |||||||TCACTGAA-TATTCGAA 229

**Figure 6**

### Physical Map of 20q13.2 Breast Cancer Amplicon

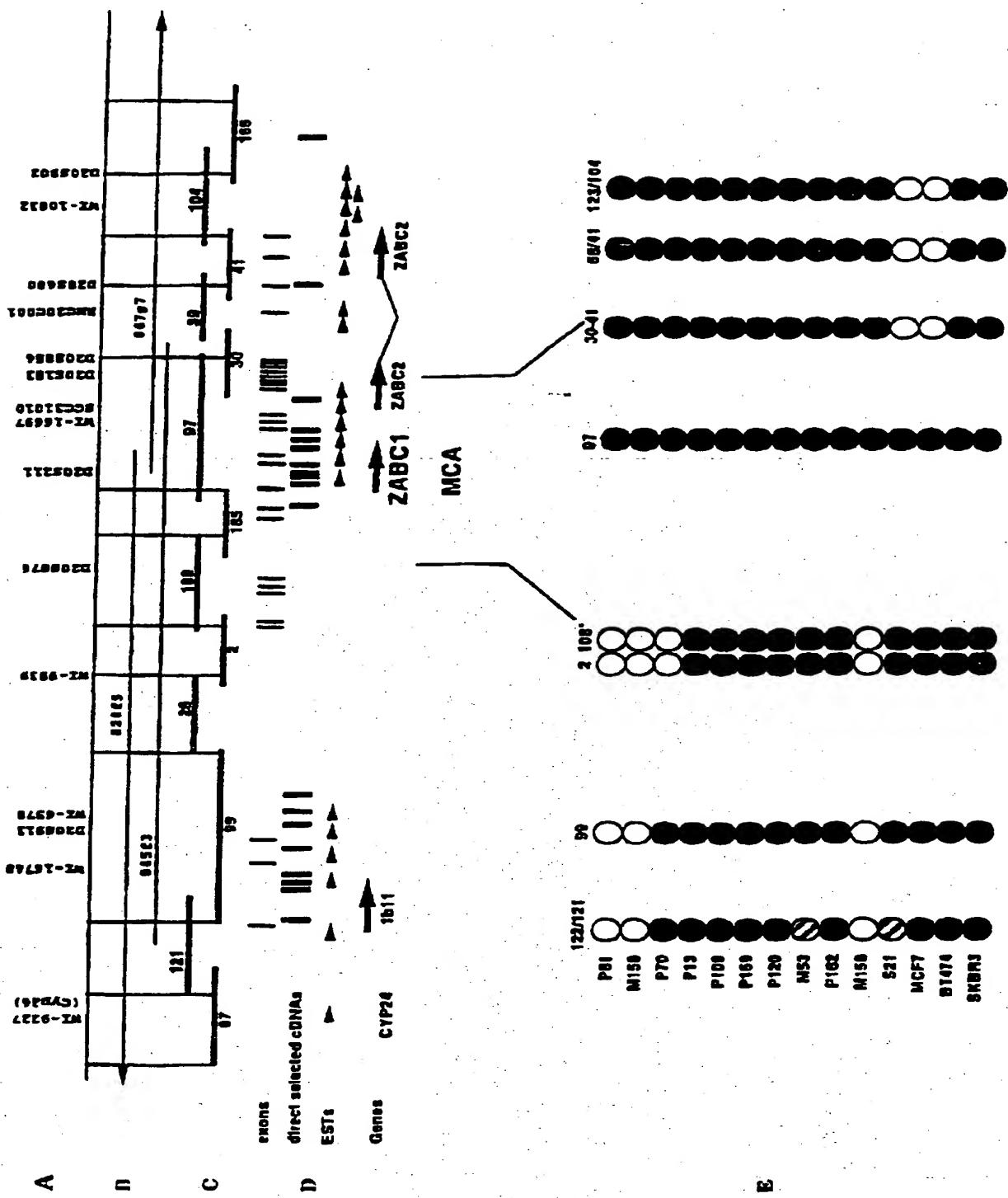


Figure 7

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/12343

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : C12N 15/11; C12Q 1/68, A61K 48/00

US CL : 536/23.1; 435/6; 515/44

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/6; 515/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MPSRCH

search terms: 20q13 amplicon, ZABC1

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document; with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHORT et al. Lambda ZAP: a bacteriophage lambda expression vector with in vivo excision properties. Nucleic acids Res. 1988, Vol. 16, No. 15, pages 7583-7600. See entire document.	1, 5
X	WO 93/25671 A1 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION) 23 December 1993. See entire document.	1, 5
X	OKUBO et al. An expression profile of active genes in human colonic mucosa. DNA Res. 1994, Vol. 1, pages 37-45. See entire document.	1, 11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance		
*E* earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*O* document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
*P* document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

15 AUGUST 1997

Date of mailing of the international search report

15 SEP 1997

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

MINH-TAM DAVIS

Facsimile No. (703) 305-3230

Telephone No. (703) 308-0916

Form PCT/ISA/210 (second sheet)(July 1992)\*

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US97/12343

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAWLAK et al. Characterization of a large population of mRNAs from human testis. Genomics. 1995, Vol. 26, pages 151-158. See entire document.	1, 13
X	WO 93/24514 A (MITOTIX) 09 December 1993. See entire document.	1, 15
X -- Y	MORRIS et al. Entire ABL gene is joined with 5'-BCR in some patients with Philadelphia-positive leukemia. Blood. 15 August 1991, Vol. 78, No. 4, pages 1078-1084. See entire document.	1, 15 ----- 25, 36
X	HOROWITZ et al. The human glucocerebrosidase gene and pseudogene: Structure and evolution. Genomics. 1989, Vol. 4, pages 87-96. See entire document.	1, 15
X	GORBULEV et al. Organization and chromosomal localization of the gene for the human bombesin receptor subtype expressed in pregnant uterus. FEBS Lett. 1994, Vol. 340, pages 260-264. See entire document.	1, 20

Form PCT/ISA/210 (continuation of second sheet)(July 1992)\*

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US97/12343

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1.  **Claims Nos.:**  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  **Claims Nos.:**  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  **Claims Nos.:**  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-42, 44-46

**Remark on Protest**  

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)